THE INDIAN JOURNAL OF PHYSIOLOGY AND ALLIED SCIENCES

JANUARY, JULY 1949

Published by
THE PHYSIOLOGICAL SOCIETY OF INDIA
CALCUTTA

Price Rs. 16 per volume

"The path of modern organ physiology is straight and clear, and we are not far from a complete understanding of life as an association of organs. But the organ is an assembly of cells and its properties and activities are dependent on the properties and activities of its component cells. Organ physiology has therefore, so to speak, begun its study from the midst of life; the beginning, the basis of life is in the cell."

—I. Pavlov, 1897.

The Indian Journal of
Physiology and Allied Sciences
Vol. III, Nos. 1 & 2, 1949.

CHEMOTHERAPEUTIC ACTION & TOXICITY OF DIMIDIUM BROMIDE

By

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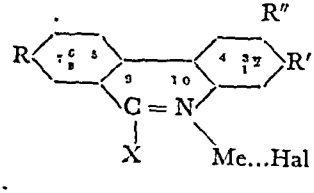
Chemotherapeutic activity of phenanthridinium compounds against trypanosome infection in animals has been studied by a number of workers [Browning and Calver (1943), Calver (1945), Carmichael and Bell (1943), Fulton and Yorke (1943), Wien (1946) and Browning, Calver, Leckie, Walls (1946)]. Some of the compounds of this series have shown marked activity in infected mice. The action is specific; thus S 1554 (3-carbethoxy-amino-9-carbethoxy amino phenyl-10-methylphenanthridinium chloride) and S 1582 (2:7 dicarbethoxy-amino-9:10-dimethylphenanthridinium methosulphate) which influence *T. cruzi* infection do not act on *T. brucei* and *T. congolense* except in high doses; on the other hand 1553 (2:7-diamino-9-phenyl-10-methylphenanthridinium bromide) and 897 (7:amino-9-*p*-amino-phenyl-10-methylphenanthridinium chloride) have been shown to be highly potent against *T. congolense*. Again, the diamidine compounds, which are highly effective against *T. equiperdum* and *T. rhodesiense*, show little activity against *T. congolense* in contrast to phenanthridinium compounds which are ineffective against *T. equiperdum* except in high doses.

In the present paper the chemotherapeutic activity of Dimidium bromide (1553) has been studied against *T. evansi* and *T. equiperdum* infections in mice. The toxicity of the compound has also been determined. The structural formula of the compound is shown from the general formula given below, where R=R′=NH_2, R″=H, X=⬡



EXPERIMENTAL

Groups of albino mice from an inbred laboratory strain weighing between 15 gm. and 25 gm. were used. Chemotherapeutic activity was determined against *T. evansi* and *T. equiperdum* infections. Citrate saline suspension of the organism was prepared from the heart blood of an infected mouse, containing 7,000 organisms per cu. mm., approximately. Mice for the experiments were injected intraperitoneally with 0·5 c.c. of this suspension and those animals showing *moderate infection (100,000 to 500,000* organisms per cu. mm.*)* in the peripheral blood after 48 hours of injection were used. The compound was injected subcutaneously the concentration used being so adjusted that not more than 0·3 c.c. and not less than 0·1 c.c. was used for each animal. Wet preparations of blood from the tail were examined periodically, a mouse being regarded as negative if no trypanosomes were found in at least 20 microscopic fields. The drug was considered effective if the peripheral blood was free from organisms for 72 hours and curative if free for 28 days. For toxicity tests, mice weighing between 15 and 20 gm. were used. Intravenous as well as subcutaneous injections were given, the percentage of the solution used being 1% for subcutaneous injection and 0·2% for intravenous injection. Mortality was observed for 72 hours after injection and the LD 50 was determined by Kärber's method.

RESULT

The results are shown in tables I, II and III.

TABLE I

Evaluation of the activity of Dimidium Bromide against T. evansi

Dose—mg/gm.	No. of mice	*No. of mice cleared of Trypanosomes* 72 hrs.	28 days
0·0005	4	0	—
0·001	4	0	—
0·005	4	0	—
0·01	4	0	—
0·02	4	0	—
0·03	4	2	—
0·04	4	3	1
0·05	4	4	3

TABLE II

Evaluation of the activity of Dimidium Bromide against T. equiperdum

Dose—mg/gm.	No. of mice	*No. of mice cleared of Trypanosomes* 72 hrs.	28 days
0·01	4	0	—
0·02	4	0	—
0·03	4	0	—
0·04	4	3	—
0·05	4	4	4

LD50 and toxicity levels estimated by Kärber's formula:—

The toxicity of the compound has been determined by Kärber's method, as the method is suitable for determining the toxicity of unknown substances using small groups of animals; further, previous knowledge of the relation of dose to percentage effect is not necessary with this method. A series of 7 doses were selected, each for the subcutaneous and intravenous routes; for each dose an equivalent number of male and female mice of equivalent weight (group weight for each dose) were used. The LD50 was calculated from the formula—$\text{Log LD50} = X_0 - \Sigma \frac{(p_1 + p_2)\,d}{2}$

TABLE III

Toxicity of Dimidium Bromide to albino mice

Log dose	Log equivalent mg/gm.	How injected	Observed mortality within 72 hrs. of inj.	LD50
$\bar{2}\cdot60$	0·0399	Subcutaneously	0/6	
$2\cdot65$	0·0447	do.	1/6	
$\bar{2}\cdot70$	0·0502	do.	1/6	
$\bar{2}\cdot75$	0·0563	do.	3/6	0·0656 mg/gm.
$\bar{2}\cdot80$	0·0631	do.	4/6	
$\bar{2}\cdot85$	0·0708	do.	3/6	
$\bar{2}\cdot90$	0·0795	do.	6/6	
$\bar{2}\cdot15$	0·0141	Intravenously	0/5	
$2\cdot20$	0·0159	do.	1/5	0·024 mg/gm.
$\bar{2}\cdot25$	0·0178	do.	2/5	
$\bar{2}\cdot30$	0·0200	do.	1/5	
$\bar{2}\cdot35$	0·0224	do.	2/5	
$\bar{2}\cdot40$	0·0251	do.	3/5	
$\bar{2}\cdot45$	0·0282	do.	5/5	

Discussion

A consideration of the results obtained with Dimidium Bromide shows that the compound has very little action against either *T. evansi* or *T. equiperdum*, the CD 50 figures almost approaching the toxicity figure; the therapeutic index has been found to be 1·5. Wien (1946), Browning and Calver (1943) and Calver (1945) on the other hand, have found the compound to be highly effective against *T. congolense*, the therapeutic index as obtained by Wien (1946) being 152. This difference in action on different trypanosomes clearly illustrates the specificity of action of the drug, a feature already pointed out by Walls and Browning (1945). There appears to be some relationship between the chemical structure and specificity of action, one particular type of chemical structure being responsible for action, against particular trypanosome. The subject has been discussed in some detail by Wien (1946).

In evaluating the toxicity of the drug in white mice, figures slightly differing from that of Wien (1946) have been obtained, our figures being on the higher side. LD 50 figures as obtained by us are

as follows: 0·065 mg/gm for subcutaneous route and 0·024 mg/gm for intravenous route in contrast to 0·061 mg/gm and 0·020 mg/gm respectively obtained by Wien (1946). This increased tolerance in our case, though not of high magnitude, may possibly be explained from consideration of such factors as difference in strain of animals (white mice) used in two cases and the difference in climatic conditions.

Summary

1. An evaluation of the chemotherapeutic activity of Dimidium Bromide (2:7-diamino-9-phenyl-10-methylphenanthridinium bromide) against *T. evansi* and *T. equiperdum* showed that the compound has very little action against these organisms. The chemotherapeutic index has been found to be 1·5, in contrast to 152 (Wien) for *T. congolense*.

2. The toxicity of the drug has been determined in white mice, the LD 50 figures (0·065 mg/gm and 0·024 mg/gm for subcutaneous and intravenous routes respectively) differing slightly from those of Wien (0·061 mg/gm and 0·020 mg/gm respectively).

Acknowledgment

The drug was sent to us by Dr. G. Hobday of the Boots Pure Drug Co., London and our thanks are due to him. Dr. B. B. Roy of the R. G. Kar Medical College, then on deputation at the Central Drugs Laboratory, also rendered assistance in the preliminary stages of the work.

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The Indian Journal of
Physiology and Allied Sciences
Vol. III, Nos. 1 & 2, 1949.

EFFECT OF GERMINATION, AUTOCLAVING AND DRY HEAT TREATMENT ON THE NUTRITIONAL VALUE OF *CICER ARIETINUM* L (GRAM), AND *PHASEOLUS AUREUS* ROXB (SONA MUNG)

By

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The beneficial effect of a short period of roasting on the biological value of protein of Soya bean has already been noted.[1] It has also been found that a better growth may be obtained by using cooked navy and velvet bean proteins.[2, 3] It has been suggested that these causes of improvement in the protein assimilation may be due to the destruction of some heat-labile antitryptic factor present in the raw bean.[4] Every food protein does not respond to these treatments in a similar way. It has, thus, been observed that many cereal proteins would rather be adversely affected by dry heat treatment.[5] The nutritional quality of many animal proteins are also similarly affected by prolonged heating.[6] It was considered worth while to investigate how the biological values of the pulse proteins—which are one of the main sources of protein in the Indian diet—are affected by roasting, autoclaving and germination. The present investigation is limited to the observations of the effects of these treatments on *Cicer Arietinum* L and *Phaseolus Aureus* Roxb proteins.

Hand picked grains of these pulses of uniform size and quality were selected for various types of treatments. The process of roasting was carried on sand baths, by subjecting gram to a temperature of 250°C for three minutes, and Sona mung for one minute. They were then finely pulverized and used for experiments.

For Autoclaving the pulses were throughly soaked in water and autoclaved for thirty minutes at one and half atmosphere pressure. They were then crushed and quickly dried to a constant weight at a low temperature and puverised to a fine and uniform powder.

For germination pulses were steeped in tapwater for twelve hours. The germination was carried in room temperature for about forty-

eight hours, in case of gram and twentyfour hours in case of Sona mung. The germinated beans were then crushed and quickly dried to a constant weight at a low temperature. They were then pulverised to a fine and uniform powder. Raw gram and Mung beans were also finely powdered and reduced to a constant weight.

All these samples were analysed and the percentage of protein, fat, and carbohydrate were determined. On the basis of these analysis, diets containing 5%, 10%, and 13% crude proteins from gram and mung beans were prepared. The percentage of protein in the food mixtures were checked by chemical analysis. In the standardisation period, a low nitrogen diet, containing about 4% egg powder (about 0·5% nitrogen) was employed to avoid unpalatibility and unphysiological character of absolutely protein free diet.[7]

TABLE I

Composition of low nitrogen diet for the standardisation period

Egg powder	4	gms
Butter fat	3	gms
Cane Sugar	15	gms
Starch	74·57	gms
*Vitamins and Minerals	1·43	gms
Total	100	gms

TABLE II

Composition of diets used for the determination of biological value

Diet whose source of protein is		Gram gm	Mung bean gm	Starch gm	Butter fat, Cane sugar Vitamins and Minerals
Gram Protein	Raw, at 13% level	64·87		15·70	As in Low-Nitrogen diet—(Table I)
	Do at 5% level	27·77		52·80	
	Roasted, at 13% level	58·14		22·43	
	Autoclaved, at 5% level	23·28		57·29	
	Germinated, at 5% level	22·83		57·74	
Mung Bean Protein	Raw, at 10% level		42·99	37·58	
	Roasted, at 10% level		41·02	29·55	
	Germinated, at 10% level		42·19	38·38	
	Autoclaved, at 10% level		41·84	38·73	

Full grown rats, of uniform weight and of similar age, were selected for the experiments. Each batch of rats were kept on a low nitrogen diet (Table I) for a period of six days; urine and feces

* Proper amount of vitamins in form of vitaminist tabloids and salt mixture were mixed with the diet just before the food mixtures were served to the animals.

being collected on the last three days of the experiment, for the determination of endogenous and metabolic nitrogen. The rats were then maintained with the diet containing pulse protein, for a further period of nine days. Feces and urine were preserved for analysis, for the last six days. Urine and the washings of the funnels were preserved with toluene in 0·5% sulphuric acid in refrigerator, until analysed. The feces after collection was quickly desicated, powdered and stored in a refrigerator after removing the attached hairs from them. All the nitrogen determination of diet, urine and fecal matter were done by the Micro-Kjeldahl method of Ma and Zuazaga.[8]

TABLE III

Digestibility and the Biological value of Raw gram powder at 13% protein level

Number of experiments	I	II	III
Food intake in gms	64·57	43·04	68·52
Nitrogen intake in mgms	1343	895·2	1425
Urinary nitrogen in mgms ..	640·5	443·4	572·7
Fecal nitrogen in mgms	466·5	201·9	447·6
Endogenous nitrogen in mgms ..	168·7	108·5	134·7
Metabolic nitrogen in mgms ..	356	195	258·6
Digestibility	92%	99·2%	86·8%
Biological Value	61·7	62·3	64·5

TABLE IV

Digestibility and Biological Value of Raw gram powder at 4·97% level

Number of experiments	I	II	III
Food intake in gms	88·82	63·02	69·2
Nitrogen intake in mgms	706·4	501·1	550·3
Urinary nitrogen in mgms ..	448·3	269·3	322
Fecal nitrogen in mgms	214·6	183·9	158·6
Endogenous nitrogen in mgms ..	152·6	97·1	99·1
Metabolic nitrogen in mgms ..	204·0	137·4	150·6
Digestibility	98·5%	97%	98·5%
Biological Value	57·5	62·1	58·9

TABLE V

Digestibility and the Biological Value of roasted gram at 13% protein level

Number of experiments	I	II	III	IV
Food intake in gms	58·38	56·5	57·67	79·69
Nitrogen intake in mgms ..	1214	1175	1199	1658
Urinary nitrogen in mgms ..	394·1	336·8	321·1	584·2
Fecal nitrogen in mgms ..	367·2	403·5	444·9	362·7
Endogenous nitrogen in mgms ..	155·6	106·4	126·2	168·7
Metabolic nitrogen in mgms ..	278·3	203·4	227·0	356
Digestibility	92·6%	83%	81·8%	99·6%
Biological Value	78·8	76·3	80·1	74·8

TABLE VI

Digestibility and Biological value of germinated gram protein at 5·49% protein level

Number of experiments	I	II	III	IV	V
Food intake in gms	70·32	52	67·71	51·82	68·47
Nitrogen intake in mgms ..	617·7	456·7	594·8	455·2	601·4
Urinary nitrogen in mgms ..	352·0	231·0	325·9	231·0	368·1
Fecal nitrogen in mgms	360·3	211·7	361·3	239·0	335·4
Endogenous nitrogen in mgms ..	221·0	108·2	221·0	101·3	233·3
Metabolic Nitrogen in mgms ..	203·5	171·3	186·8	218·0	213·2
Digestibility	74·6%	91·1%	70·7%	95·4%	79·6%
Biological Value	71·5	70·5	75	70·1	71·8

TABLE VII

Digestibility and Biological Value of autoclaved gram protein at 5% protein level

Number of experiments	I	II
Food intake in grams	60·88	74·88
Nitrogen intake in mgms	487·0	599·0
Urinary nitrogen in mgms	227·4	280·0
Fecal nitrogen in mgms	191·7	187·2
Endogenous nitrogen in mgms ..	97·1	99·1
Metabolic Nitrogen in mgms ..	137·4	157·9
Digestibility	88·8%	95·1%
Biological Value	69·8	68·2

TABLE VIII

Digestibility and Biological value of raw Mung bean proteins at 9·9% protein level

Number of experiments	I	II	III	IV
Food intake in gms	53·19	80·44	52·86	78·69
Nitrogen intake in mgms ..	842·6	1274·0	837·4	1246·0
Urinary nitrogen in mgms ..	564·7	699·0	451·3	683·0
Fecal nitrogen in mgms ..	332·6	585·3	389·9	479·1
Endogenous nitrogen in mgms ..	162·2	250·6	174·2	200·2
Metabolic Nitrogen in mgms ..	252·7	313·7	182·0	308·0
Digestibility	90·5%	78·6%	74·1%	86·2%
Biological Value	47·2	55·2	55·3	55·0

TABLE IX

Digestibility and Biological Value of Autoclaved Mung bean protein at 10·74% protein level

Number of Experiments	1	II	III	IV
Food intake in gms	87·19	78·59	83·17	86·25
Nitrogen intake in mgms ..	1498·0	1351·0	1430·0	1482·0
Urinary nitrogen in mgms ..	826·0	715·0	721·0	785·5
Fecal nitrogen in mgms ..	564·2	685·9	633·0	607·0
Endogenous nitrogen in mgms ..	133·2	200·0	132·4	121·6
Metabolic nitrogen in mgms ..	256·0	308·1	256·1	254·8
Digestibility	79·4%	72·1%	73·6%	76·2%
Biological Value	40	46	44·1	41·2

TABLE X

Digestibility and Biological Value of roasted Mung bean protein at 10·51% protein level

Number of experiments	I	II	III	IV	V
Food intake in gms	99·62	77·27	106·4	68·18	103·5
Nitrogen intake in mgms ..	1676·0	1300·0	1790·0	1147·0	1741·0
Urinary nitrogen in mgms ..	908·3	827·1	930·7	620·9	850·0
Fecal nitrogen in mgms	514·1	330·9	646·4	470·7	636·9
Endogenous nitrogen in mgms ..	162·2	182·4	250·6	174·2	200·2
Metabolic nitrogen in mgms ..	252·7	218·0	313·7	182·0	308·0
Digestibility	84·4%	91·3%	81·4%	74·8%	80·1%
Biological Value	47·2	45·7	53·3	47·9	53·1

TABLE XI

Digestibility and Biological Value of germinated Mung bean protein at 9·52% protein level

Number of experiments	I	II	III	IV	V
Food intake in gms	116·6	87·29	71·38	75·76	66·09
Nitrogen intake in mgms ..	1781·0	1329·0	1087·0	1154·0	1007·0
Urinary nitrogen in mgms ..	1069·0	778·7	643·6	683·9	609·0
Fecal nitrogen in mgms	691·4	518·5	512·0	505·0	365·2
Endogenous nitrogen in mgms ..	195·3	133·2	132·4	121·6	114·2
Metabolic nitrogen in mgms ..	304·2	256·0	256·1	254·8	196·2
Digestibility	78·2%	80·2%	76·5%	78·3%	83·2%
Biological Value	37·3	39·5	38·5	37·8	40

The results of experiments show, that *Cicer Arietinum* L and *Phaseolus Aureus* Roxb have not responded to various processing methods in an identical manner, though both of them belongs to the same natural order. We have investigated the occurance of anti-proteolytic enzymes, that may be present in raw beans, but have failed to establish their presence in these two varieties of beans. Moreover the absence of any remarkable change in digestibility after the drastic treatments of processing suggests the absence of such factors. It will be noted that the Biological Value of *Cicer Arietinum* L (Gram) improves on roasting, germination and autoclaving—the improvement on roasting being most remarkable. Thus the popular methods of consuming gram in form of roasted powder and after germination appear to be scientifically supported. *Phaseolus Aureus* Roxb (Mung) on the other hand has an initial lower biological value, which is not significantly altered by roasting. A slight lowering of biological value may be observed after autoclaving, but the depression is more remarkable after germination.

We have not been able to elucidate the mechanism of these changes. The alteration of either physical or chemical character or both of the protein molecules of these beans may be factors contributing to the changes in the biological value. The change in the physical nature of the protein molecule may affect the biological value by altering the rate and sequence of liberation of the essential amino acids. The chemical character of the bean proteins may be altered during germination by preferential utilisation or destruction of the essential amino acids at different rate. Both these possibilities are now receiving our attention.

Summary

(1) There is no remarkable alteration of digestibility of these beans after various processing treatments.

(2) The biological value of *Cicer Arietinum* L protein definitely improves on roasting, and also to some extent during germination and autoclaving under experimental condition.

(3) The biological value of *Phaseolus Aureus* Roxb protein is adversely affected during germination but not altered during roasting and autoclaving under the experimental condition.

(4) The biological value of *Cicer Arietinum* L is higher than that of *Phaseolus Aureus* Roxb.

(5) No antiproteolytic factor could be traced in these beans.

The authors are grateful to The Lady Tata Memorial Trust for encouraging this investigation by offering a scholarship to one of the authors and Dr. B. B. Sarkar for taking a kind interest in the work.

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The Indian Journal of
Physiology and Allied Sciences
Vol. III, Nos. 1 & 2, 1949.

A SIMPLE METHOD OF ESTIMATION OF TOTAL CARBOHYDRATES IN PULSES AND CEREALS*

By

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In the estimation of total carbohydrates the substances are first hydrolysed with acid to convert the complex carbohydrates into monosaccharides which are quantitatively estimated either with Benedict's reagent or with Fehling's solution. While Woodman[1] recommends three hours hydrolysis with 10 per cent acid and McCance and Shipp[2] prefer four hours hydrolysis with 1 per cent acid, Association of Official Agricultural Chemists[3] and McCance, Widdowson and Shakleton[4] advocate enzymic hydrolysis with takadiastase. The above procedures are all time consuming.

After hydrolysis the hydrolysates become coloured and they also contain protein both of which interfere with the estimation of monosaccharides by the different wellknown methods. A simple method of estimation of total carbohydrates in pulses and cereals has, therefore, been developed.

EXPERIMENTAL

Hydrolysis of the food-stuffs with acids of different strengths for varying periods:

Samples of one gram of different pulses and cereals were taken in 250 c.c. conical flaks and 100 c.c. of 1, 2·5, 5, 7·5 and 10 per cent hydrochloric acids were added. A small funnel was placed at the mouth of the flask in order to prevent the loss of water so as to maintain the concentration of the acid in the flasks. The flasks were kept in the boiling water baths for periods varying between one half and four hours. The hydrolysates thus obtained were neutralised with sodium carbonate or caustic soda solution and the colour of the

* Read before a meeting of the Physiological Society of India held on May 10, 1949.
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hydrolysate was found to change with the change of the pH of the solution.

Removal of proteins and colour in the acid hydrolysates:

The neutralized hydrolysates were treated with the following reagents as detailed below:—

(a) *Basic Lead Acetate:* A 10 per cent solution of basic lead acetate was added drop by drop with constant shaking of the flask until the precipitation was complete. The excess of lead was removed by the use of saturated solution of sodium phosphate.

(b) *Mercuric Chloride:* 10 c.c. of a 1 per cent solution of mercuric chloride was added with constant shaking.

(c) *Tungstic acid:* 7 c.c. of a 10 per cent solution of sodium tungstate was added following by 10 c.c. of 2/3 N sulphuric acid.

(d) *Zinc sulphate and sodium hydroxide:* 10 c.c. of a 20 per cent solution of zinc sulphate followed by 7 c.c. of a 2N sodium hydroxide were added to the neutralized hydrolysate.

(e) *Zinc sulphate and barium hydroxide:* 20 c.c. of 0·3 N barium hydroxide solution followed by 10 c.c. of a 10 per cent solution of zinc sulphate were added into the contents of the flask.

The contents in the flasks after all the above procedures were brought to a definite volume with water and filtered.

Estimation of sugar:

The reducing sugar in the neutralised hydrolysate, before and after removal of the interfering substances, was estimated by the following methods: (*a*) Lane and Eynon's modification of Fheling's method using methylene blue as internal indicator; (*b*) Benedict's quantitative method and Hans' modification of Hagedorn and Jensen's method.[5, 6, 7]

Total reducing sugars obtained after hydrolysis of seeds of *Phaseolus Mungo* with acids of different strengths for varying periods are given in Table 1. Reducing sugars obtained after hydrolysis of

TABLE 1

Total Reducing Sugars Present in gm. per 100 gm. of Phaseolus Mungo Calculated as Glucose hydrolysed with acids of different strengths for varying periods.

Strength of acid	*Period of hydrolysis in hours*						
	½	1	1½	2	2½	3	4
1% ..	8·5	10·6	12·0	19·5	32·6	48·2	56·5
2·5% ..	12·5	20·0	2·9	39·7	48·9	56·5	56·5
5% ..	16·9	28·9	40·0	49·0	56·6	56·6	50·5
7·5% ..	20·6	39·2	56·5	56·5	56·5	52·2	50·6
10% ..	35·5	56·5	56·5	56·5	52·5	50·5	45·9

wheat flour with 10 per cent hydrochloric acid for one hour both before and after removal of the interfering substances by the various methods are given in Table 2.

TABLE 2

Total Reducing Sugars Present in 100 gm. of Wheat Flour, hydrolysed with 10 per cent. of Hydrochloric Acid for One Hour, in gm.

Reagents used to remove the interfering substances	Before Removal of interfering substances		After Removal of interfering substances		
	Lane & Eynon Method	Benedict Method	Lane & Eynon Method	Benedict Method	Hans Method
Lead acetate ..	70·2	67·9	71·5	71·6	71·6
Mercuric chloride ..	70·2	67·9	71·6	71·6	71·6
Sodium tungstate and sulphuric acid ..	70·2	67·2	71·5	71·5	71·5
Zinc sulphate and sodium-hydroxide ..	70·2	67·8	72·1	72·2	72·1
Zinc sulphate and barium-hydroxide ..	70·2	57·5	72·2	72·1	72·1

Discussion

It has been found that mercuric chloride although removes the protein in the hydrolysate could not remove the colour. Lead removes all the interfering substances but the process of removal of the excess of lead is difficult and also requires more time. Tungstic acid, zinc sulphate and sodium hydroxide and zinc sulphate and barium hydroxide remove the colour as well as the protein efficiently. Of these reagents zinc sulphate and barium hydroxide is least expensive and the best reagent in our conditions of experiment.

Table 1 shows that complete hydrolysis of the carbohydrates into reducing sugars is effected after one hour when 10 per cent hydrochloric acid is used. As the concentration of the acid is decreased more time becomes necessary for the complete hydrolysis and with 1 per cent acid hydrolysis for four hours is necessary as was observed by McCance and Shipp.[2] If the hydrolysis is continued for more than two hours with 10 per cent acid there is some loss in the total carbohydrate content. Hydrolysis with 10 per cent should not, therefore, be contiued for three hours as suggested by Wood.[1]

If the sugar in the acid hydrolysate is estimated without the removal of the interfering substances low values are obtained. When the interfering substances are removed by any of the methods described and then sugar is estimated with any of the methods presented, more or less constant result is obtained. As the estimation of reducing sugar in the clear filtrate with Benedict's method is the easiest and least time consumng of all the methods this method is recommended for the estimation of reducing sugars.

In order to estimate the total carbohydrates in pulses and cereals it is suggested that the material should be hydrolysed for one hour with 10 per cent hydrochloric acid. The solution should be neutralized with 40 per cent sodium hydroxde and the colour and protein should be removed with barium hydroxide and zinc sulphate solutions. The sugar in the clear filtrate should be estimated with Benedict's quantitative reagent.

Summary

1. Samples of pulses and cereals were hydrolysed for varying periods with acids of different strengths. It was observed that complete hydrolysis of the carbohydrates was effected in one hour when the food-stuff was hydrolysed with 10 per cent hydrochloric acid.

2. Different reagents were used for the removal of the colour and the protein in the hydrolysate which interfere with the estimation of reducing sugars. It was observed that zinc sulphate with barium hydroxide could remove the interfering substances most efficiently.

3. When the reducing sugar was estimated without the removal of the interfering substances a lower sugar value in the sample was obtained.

4. Of the different methods of estimating reducing sugars the method of Benedict was found most suitable.

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The Indian Journal of
Physiology and Allied Sciences
Vol. III, Nos. 1 & 2, 1949.

THE ACTION OF PROGESTERONE ON THE FEMALE GENITAL SYSTEM OF JUVENILE PIGEONS

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(With one plate)

INTRODUCTION

A number of workers have demonstrated that prolonged injection of crude corpora lutea extracts or progesterone inhibit the estrus cycle of rats and mice (Parkes and Bellerby, 1927; Hisaw *et al*, 1928; Haterius and Piffner, 1929; Selye *et al*, 1936; Phillips, 1937). Dempsy (1937) showed that progesterone prevent preovulatory swelling of the Graafian follicles in the guineapig. Inhibition of ovulation that normally follows estrus has been reported by Makepiece *et al* (1937). In all these experiments prolonged injections of progestin or progesterone were made over long periods of time. In a series of studies Everett (1940, 1944 and 1948) has shown that a single injection of this hormone in relatively high doses has ovulation-inducing effect in normal rats.

Pearl and Surface (1914) reported ovulation-inhibitory action of corpus luteum extract in the fowl. Recently, Rothchild and Fraps (1949) reported that ovulation cannot be induced in the fowl with progesterone in the birds whose pituitaries are low in ovulating potency or in hypophysectomized birds, but this hormone does induce ovulation in normal or sham hypophysectomized birds. These authors assume that progesterone induces a release of ovulating hormone from the hypophysis. In adult pigeons inhibition of ovulation have been noted following progesterone treatment (Riddle *et al*, 1941).

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From a review of the works cited above it is evident that the action of progesterone on the female genital system of juvenile birds and mammals has not hitherto been reported. It seemed desirable therefore, to contribute the present observations since an attempt has been made here for the first time to study the action of progesterone on the female genital system of juvenile pigeons.

Procedure

Female pigeons, 80 days old were involved in this investigation. The birds were housed in cages and maintained under uniform husbandry conditions throughout the duration of the experimental period. The initial body weight of each bird was taken at the commencement of the experiment and the final weight was noted on the day of autopsy.

Progesterone in sterile sesame oil was administered intramuscularly. The daily injections (0·5 mgm.) were made into the breast muscles and continued for a period of 30 days (March 8 to April 6, 1949). The site of injection was altered from day to day on the right and left sides of the breast. Uninjected birds served as the controls.

All the birds were autopsied on the day following the final injections. The ovary and the oviduct were carefully dissected out, weighed to the nearest mgm., and finally fixed in alcoholic Bouin's fluid for histological studies. Serial sections, 8 microns thick were prepared by the paraffin method and stained with Heidenhains iron hematoxylin followed by eosin.

Experimental Results

Macroscopic observations: Examinations at autopsy revealed pronounced inhibitory effects of progesterone treatment on the genital system of the experimental females. A perusal of table 1 will clearly indicate that the absolute and relative weights of the ovary and the oviduct of the progesterone recipients were significantly lower as compared with those of the ovary and the oviduct of the control specimens.

Table I—Oviducal and Ovarian Weights in Control and Progesterone-treated Pigeons

Treatment	Number of birds	Oviducal weight		Ovarian weight		Body weight at autopsy
		Absolute	Relative†	Absolute	Relative	
		Mgm.	Percent	Mgm.	Percent	Gm.
Untreated controls	10	501·2±2·6*	·0002	280·5±1·8	·0001	198·5±2·2
Progesterone treated	10	302·1±1·7	·0001	170·2±1·2	·00008	203·4±1·9

The oviduct of the treated birds was a pale and non-tortuous structure. The oviducal ligaments were inconspicuous structures and the bundles of muscle fibres characteristic of the ligaments were

* Standard error of the mean.

† Organ weight expressed as percentage of body weight.

used in this study. Thanks are due to Mr. P. C. Pathak for taking the photo-micrographs which illustrate this paper.

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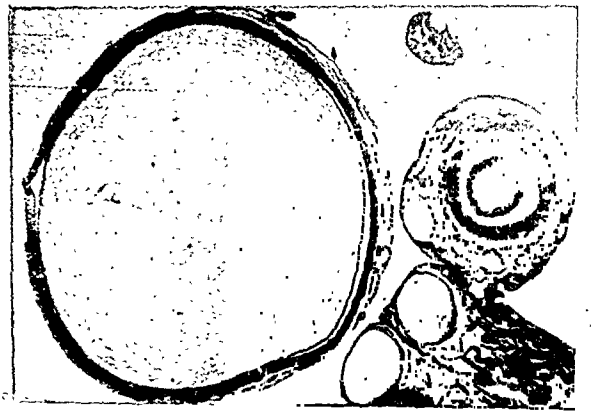
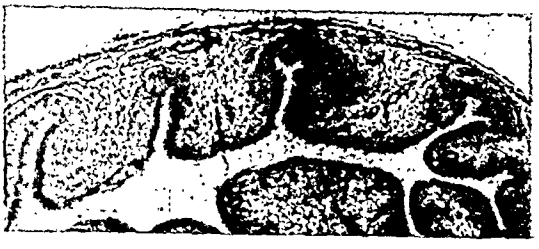
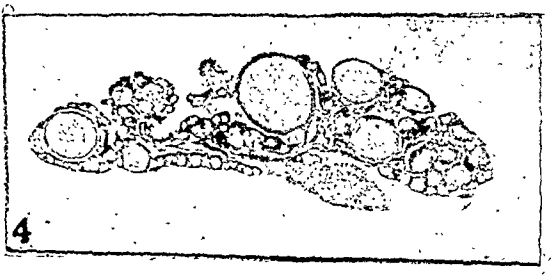
EXPLANATION OF FIGURES

Fig. 1. Photomicrograph of a portion of the transverse section through the oviduct of a control pigeon (X 30). Note the marked glandular development.
Fig. 2. Photomicrograph of the section through the ovary of a control specimen (X 20).
Fig. 3. Photomicrograph of a portion through the oviduct of a progesterone treated bird (X 30). Note the absence of the tubular glands.
Fig. 4. Photomicrograph of the section through the ovary of a progesterone treated female (X 20). Compare with Fig. 2.


1






4

EFFECTS OF ULTRAVIOLET AND INFRARED RADIATION ON THE BLOOD OF YOUNG ANIMALS

By

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Therapeutic use of ultraviolet light for cure of rickets, osteomalacia, etc. and that of infrared rays for their local effects in relieving pain have been much in practice in recent times. The infrared rays have in general a much better penetrating power than the ultraviolet rays which are capable of penetrating through only a very thin layer of skin or mucous membrane. The former produces local redness also, immediately after the exposures, while the latter can produce erythema only after long exposures (5 to 8 hours) with important changes [Colwell and Russ[1]]. Riedel[2] also has shown that rays of very small penetrating power as the ultraviolet rays have distinct effects on the blood of the experimental animals. Many other investigators have examined blood of animals before, during and after exposures and although they are agreed that there are important changes in the constitution of the blood, the precise nature and extent of such changes are not yet fully known, as has been expressed by Aitkin[3] thus, "a very distinct effect on the results of irradiation has been shown by many observers but very contradictory results have been obtained." So with a view to throw further light on the effects of these physical therapeutic agents on the blood picture of young animals after short and repeated exposures, the present investigation was taken up.

Experimental

Two batches of young rabbits, each belonging to the same litre and about two months old, were used for this experiment. In the first batch two animals were subjected to ultraviolet irradiation from a mercury quartz vapour lamp, while the third served as a control. In the second batch three animals were exposed to infrared rays from an infrared irradiation lamp, while the fourth was kept as a control. The ultraviolet exposures were repeated at 2-3 days' intervals while the infrared irradiation was repeated on alternate days, as a rule. In both the cases the exposures lasted for one hour only at a time. The experiment started after a preliminary examination of blood of all the animals in all aspects, *viz.*, total and differential counts, hæmoglobin percentage, Arneth index, platelet and reticulocytic counts, coagulation time and fragility test for red blood corpuscles. After each irradiation all these counts and tests were repeated for the exposed animals as also for their controls. The ultraviolet experiment lasted for two and half months while the infrared investigation lasted for five weeks only, as two animals subjected to irradiation suddenly died one after another, of pneumonia after 20 and 22 days respectively from the commencement of the experiment, so that for the remaining period only one animal of the second batch was subjected to the infrared rays.

The following tables show the changes in the blood picture in its various aspects, of the two groups of experimental animals as compared to their controls.

TABLE 1

Showing increase in the weight and blood changes in animals I & II after EXPOSURES TO ULTRAVIOLET RADIATION as compared to those of No. III as control.

Nos. of Exposures	No. of Animals	Hæmoglobin	Weight in lbs.	Number per c.mm. R.B.C. (Millions)	Number per c.mm. W.B.C.	Number per c.mm. Platelets (thousands)	Polymorphs	Arneth. index.	Lymphocytes	Eosinophils	Monocytes	Basophils	Reticulocytes %	Coagulation Time (minutes)
Normal	I	1·75	80	4·5	8,750	200	34	2, 34, 44, 18, 2	64	1	1	—	2	2·5
	II	1·10	75	3·7	7,500	200	38	0, 22, 46, 22, 10	61	1	—	—	2	2·5
	III	1·00	65	3·3	6,875	200	30	2, 34, 44, 16, 4	70	—	—	—	3	2·5
1.	I	1·75	80	4·5	8,125	200	40	2, 34, 44, 18, 2	60	—	—	—	2	2·5
	II	1·10	75	3·72	7,500	200	39	0, 20, 48, 22, 10	61	—	—	—	2	2·5
	III	1·00	65	3·33	6,875	200	30	2, 34, 45, 15, 4	70	—	—	—	3	2·5
2.	I	1·75	80	4·6	8,750	200	45	1, 24, 54, 20, 1	54	—	1	—	2	2·5
	II	1·10	75	3·8	7,500	200	40	0, 20, 48, 22, 10	60	—	—	—	2	2·5
	III	1·00	65	3·33	6,875	200	30	2, 34, 44, 16, 4	70	—	—	—	3	2·5
3.	I	2·14	85	5·0	9,375	200	45	0, 20, 58, 20, 1	55	—	—	—	3	2·25
	II	1·10	80	4·2	8·125	200	40	0, 18, 50, 22, 10	60	—	—	—	2	2·5
	III	1·00	65	3·84	6,875	200	30	2, 34, 46, 16, 4	70	—	—	—	3	2·5
4.	I	1·14	85	5·0	9,375	220	45	0, 19, 60, 20, 1	55	—	—	—	3	2·25
	II	1·10	80	4·3	8,125	220	40	0, 18, 50, 22, 10	60	—	—	—	3	2·25
	III	1·12	65	3·38	6,875	200	30	2, 34, 47, 13, 4	70	—	—	—	3	2·5
5.	I	2·14	90	5·2	10,000	220	45	0, 19, 60, 20, 1	55	—	—	—	3	2·25
	II	1·28	85	4·5	8,750	220	40	0, 18, 50, 22, 10	60	—	—	—	3	2·25
	III	1·12	65	3·39	6,875	200	30	2, 34, 47, 13, 4	70	—	—	—	3	2·5
6.	I	2·14	95	5·5	10,000	220	45	0, 16, 62, 20, 2	55	—	—	—	3	2·25
	II	1·28	90	4·7	8,750	220	40	0, 16, 52, 22, 10	60	—	—	—	3	2·25
	III	1·12	67	3·4	6,875	200	30	2, 34, 47, 12, 5	70	—	—	—	3	2·5
7.	I	2·14	95	5·5	10,000	220	45	0, 16, 62, 20, 2	55	—	—	—	3	2·25
	II	1·28	90	4·8	8,750	220	40	0, 14, 54, 20, 12	60	—	—	—	3	2·25
	III	1·12	67	3·4	6,875	200	30	2, 34, 48, 11, 5	70	—	—	—	3	2·5
8.	I	2·14	100	5·7	10,000	220	45	0, 16, 62, 20, 2	55	—	—	—	3	2·25
	II	1·28	95	5·0	8,750	220	40	0, 14, 54, 20, 12	60	—	—	—	3	2·25
	III	1·25	70	3·56	6,875	200	30	2, 34, 48, 11, 5	70	—	—	—	3	2·5
9.	I	2·14	100	5·8	10,000	220	45	0, 10, 64, 24	55	—	—	—	3	2·25
	II	1·28	95	5·2	8,750	220	40	0, 14, 56, 18, 12	60	—	—	—	3	2·25
	III	1·25	70	3·56	6,875	200	30	2, 34, 43, 11, 5	70	—	—	—	3	2·5
10.	I	2·14	100	5·8	10,865	220	45	0, 10, 64, 26, 0	60	—	—	—	3	2·25
	II	1·28	100	5·4	8,750	220	40	0, 14, 56, 18, 12	60	—	—	—	3	2·25
	III	1·25	70	3·59	6,875	200	30	2, 36, 46, 13, 5	70	—	—	—	3	2·5
11.	I	2·14	100	5·9	10,865	220	45	0, 10, 64, 26, 0	55	—	—	—	3	2·25
	II	1·28	100	5·4	9,375	220	40	0, 14, 50, 24, 12	60	—	—	—	3	2·25
	III	1·32	70	3·65	6,875	200	30	2, 36, 46, 13, 5	70	—	—	—	3	2·5
12.	I	2·14	100	5·9	11,750	220	45	0, 10, 64, 26, 0	55	—	—	—	3	2·25
	II	1·28	100	5·4	9,375	220	40	0, 14, 50, 26, 10	60	—	—	—	3	2·25
	III	1·32	70	3·65	7,520	200	35	2, 36, 46, 13, 5	65	—	—	—	3	2·5
13.	I	2·14	100	5·95	11,750	220	45	0, 10, 64, 26, 0	55	—	—	—	3	2·25
	II	1·28	100	5·5	9,375	220	42	0, 10, 50, 30, 10	58	—	—	—	3	2·25
	III	1·32	70	3·7	7,520	200	35	2, 36, 46, 13, 5	65	—	—	—	3	2·5
14.	I	2·14	100	5·95	11,750	220	45	0, 10, 64, 26, 0	55	—	—	—	3	2·25
	II	2·28	100	5·52	9,375	220	42	0, 10, 50, 30, 10	58	—	—	—	3	2·25
	III	1·37	70	3·72	7,520	200	35	2, 36, 46, 13, 5	65	—	—	—	3	2·5
15.	I	2·14	105	6·0	11,750	230	45	0, 8, 66, 26, 0	55	—	—	—	3	2·25
	II	1·30	100	5·52	9,375	230	42	0, 10, 50, 32, 8	58	—	—	—	3	2·0
	III	1·40	70	3·75	7,520	200	35	2, 36, 46, 13, 5	65	—	—	—	3	2·5
16.	I	2·14	105	6·0	12,500	230	45	0, 8, 64, 27, 0	55	—	—	—	3	2·0
	II	1·30	100	5·52	9,375	230	42	0, 10, 50, 32, 8	58	—	—	—	3	2·0
	III	1·40	75	3·75	7,520	200	35	2, 36, 47, 14, 5	65	—	—	—	3	2·5
17.	I	2·14	105	6·1	12,500	230	45	0, 8, 62, 29, 0	55	—	—	—	3	2·0
	II	1·30	100	5·52	9,375	230	42	0, 10, 50, 32, 8	58	—	—	—	3	2·0
	III	1·40	75	3·75	7,520	200	35	2, 36, 47, 14, 4	65	—	—	—	3	2·0

TABLE 2

Showing increase in weight and blood changes in animals 1, 2 & 3 after Exposures to Infrared Radiation as compared to those of No. 4 as a control.

Nos. of Exposures		Weight in lbs.	Hb. %	Number per c.mm. R.B.C. Millions	W.B.C.	Platelets thousands	Poly. %	Arneth count %	Lympho. %	Eosino. %	Reticulocytes %	Coagulation Time Minutes
Normal	1	1·70	80	4·6	9,375	200	36	2, 32, 46, 18, 2	63	1	2	2·5
	2	1·10	70	3·7	8,125	200	38	9, 28, 45, 16, 2	61	1	2	2·5
	3	1·20	75	3·8	7,500	200	39	10, 25, 45, 18, 2	60	—	2	2·5
	4	1·06	65	3·4	7,520	200	30	4, 30, 42, 18, 4	70	—	3	2·5
I	1	1·70	80	4·6	9,375	200	37	2, 32, 44, 20, 2	62	1	2	2·5
	2	1·10	70	3·8	8,125	200	38	9, 28, 50, 16, 2	61	1	2	2·5
	3	1·20	75	3·8	7,500	200	39	10, 25, 45, 18, 2	60	—	2	2·5
	4	1·06	65	3·4	7,500	200	30	4, 30, 42, 18, 4	70	—	3	2·5
II	1	1·70	80	4·7	10,000	200	39	5, 29, 49, 15, 3	61	—	2	2·5
	2	1·10	70	3·9	8,125	200	40	4, 28, 50, 16, 2	60	1	2	2·5
	3	1·20	75	3·8	7,500	200	40	10, 25, 45, 18, 2	59	1	2	2·5
	4	1·06	65	3·4	7,520	200	30	4, 30, 42, 18, 4	70	—	3	2·5
III	1	1·81	80	4·9	10,000	200	42	3, 20, 60, 14, 3	57	1	2	2·5
	2	1·18	75	4·2	8,125	200	40	4, 28, 50, 16, 2	60	1	2	2·5
	3	1·12	75	3·8	7,500	200	40	8, 25, 47, 18, 2	69	1	2	2·5
	4	1·06	65	3·42	7,500	200	30	4, 30, 44, 19, 3	70	—	3	2·5
IV	1	1·81	85	4·9	10,000	220	41	3, 18, 62, 14, 3	58	1	2	2·5
	2	1·81	75	4·3	8,750	200	42	4, 28, 50, 16, 2	57	1	2	2·5
	3	1·20	80	3·98	8,125	200	40	8, 23, 47, 18, 2	59	1	2	2·5
	4	1·06	65	3·43	7,520	200	30		70	—	3	2·5
V	1	1·81	85	4·9	10,000	220	43	3, 18, 62, 14, 3	57	—	2	2·25
	2	1·18	80	4·4	9,375	200	45	2, 22, 56, 18, 2	55	1	2	2·5
	3	1·20	80	4·2	8,125	200	42	6, 21, 51, 20, 2	57	1	2	2·5
	4	1·20	65	3·43	7,520	200	30	2, 34, 47, 13, 4	70	—	3	2·5
VI	1	1·81	90	5·0	10,000	220	45	3, 16, 60, 18, 3	55	—	3	2·25
	2	1·18	85	4·5	9,375	220	45	2, 20, 54, 22, 2	55	1	3	2·25
	3	1·30	80	4·2	8,125	210	42	6, 20, 52, 20, 2	58	1	3	2·25
	4	1·20	65	3·43	7,520	200	30	2, 34, 47, 12, 5	70	—	3	2·5
VII	1	1·81	90	5·0	10,625	220	45	3, 16, 60, 18, 3	55	—	3	2·25
	2	1·18	90	5·0	9,375	220	48	2, 6, 54, 26, 2	52	1	3	2·25
	3	Died of pneumonia										
	4	1·20	65	3·56	7,520	200	30	2, 34, 48, 11, 5	70	—	3	2·5
VIII	1	1·81	95	5·4	10,625	220	45	3, 14, 60, 22, 3	55	—	3	2·25
	2	Died of pneumonia										
	4	1·4	68	3·56	7,520	200	30	2, 34, 48, 11, 5	70	—	3	2·5
IX	1	1·81	95	5·5	10,625	220	45	3, 14, 60, 22, 3	55	—	3	2·25
	4	1·40	68	3·59	7,520	200	30	2, 34, 48, 11, 5	70	—	3	2·5
X	1	1·81	95	5·5	10,625	220	45	3, 14, 60, 22, 3	55	—	3	2·25
	4	1·40	70	3·6	7,520	200	30	2, 34, 48, 11, 5	70	—	3	2·5
XI	1	1·81	95	5·6	10,625	220	45	3, 14, 60, 22, 3	55	—	3	2·25
	4	1·5	70	3·6	7,520	200	30	2, 34, 48, 11, 5	70	—	3	2·5
XII	1	1·84	100	5·78	10,625	220	45	3, 14, 55, 27, 1	55	—	3	2·25
	4	1·5	70	3·6	7,520	200	35	2, 36, 46, 13, 3	65	—	3	2·5
XIII	1	1·84	100	5·8	11,250	230	45	3, 14, 55, 27, 1	55	—	3	2·25
	4	1·60	70	3·6	7,520	200	35	2, 36, 46, 13, 5	65	—	3	2·5
XIV	1	1·84	100	5·82	11,250	230	45	3, 14, 56, 26, 1	55	—	3	2·25
	4	1·60	70	3·6	7,520	200	35	2, 36, 46, 13, 5	65	—	3	2·5

SUMMARY OF TABLE I (ULTRAVIOLET EXPERTMENT)

Weight & Blood-picture	*Animals exposed to U-V Rays*		*Normal Control*
	Animal I	Animal II	Animal III
Weight gained ..	0·5 lbs.	0·25 lbs.	0·5 lbs.
Hæmoglobin ..	+25%	+30%	+10%
R.B.C. (per c.mm.)	+1·8 million	+2·2 millions	+0·45 million
W.B.C. (per c.mm.)	+3750	+3750	+675
Polymorph ..	+12%	+4%	+5%
Arneth index ..	Marked shift to right	shift to right	no shift
Lymphocytes ..	−10%	−6%	−5%
Mononuclears, Eosinophils, Basophils ..	No change	No change	No change
Platelets (per c.mm.)	+30,000	+30,000 per c.mm.	No change
Reticulocytes ..	+2%	+1%	No change
Coagulation time ..	−½ min.	−½ min.	No change
Fragility ..	No change	No change	No change

SUMMARY OF TABLE II (INFRARED EXPERIMENT)

Weight & Blood-picture	*Exposed to Infared irradiation*				*Normal control*	
	No. 1 (35th day)	No. 1 (21st day)	No. 2 (21st day)	No. 3 (21st day)	No. 4 (35th day)	No. 4 (21st day)
Weight Gain ..	+0·15 lb.	+0·11 lb.	+0·08 lb.	+0·1 lb.	+0·4 lb.	+0·34 lb.
Hæmoglobin ..	+20%	+15%	+20%	+5%	+10%	+3%
R.B.C. (per c.mm.) ..	+1·22 millions	+0·8 mil.	+1·3 mil.	+4 mil.	+0·45 millions	+0·26 millions
W.B.C. „ ..	+2000	+1250	+1250	+625	+675	+20
Polymorph ..	+9%	+9%	+10%	+3%	+5%	+0%
Arneth index ..	Slight shift to right				No shift	
Lymphocyte ..	−8%	−8%	−9%	−2%	−5%	No change
Mononuclear, Eosinophils, Basophils ..	No change	No change	No change	No change	No change	No change
Platelets (per c.mm.)	+30,000	+20,000	+20,000	%10,000	No change	No change
Coagulation time	−½ min.	−¼ min.	−¼ min.	−¼ min.	No change	No change
Fragility of R.B.C. ..	No change	No change	No change	No change	No change	No change

Discussion

From the above tables it may be noted that ultraviolet or infrared irradiation has practically no effect on the weight of the animals.

As regards red blood corpuscles, ultraviolet rays increased their number and the percentage of hæmoglobin also enormously (by 1·8 to 2·2 millions per c.mm. and 25 to 30 per cent respectively). There was also a slight increase in the reticulocytic count too, but the fragility of the red cells was not affected in any way.

After exposures to infrared rays, two out of three animals exhibited some increase of red cell count as also of the percentage of hæmoglobin, while the third did not show any marked increase. There was also a slight increase in the number of reticulocytes in all the three experimental animals as compared to the normal, although there was no change in the fragility of the red cells.

As to the white blood corpuscles, the ultraviolet irradiation increased the total count by about 4,000 per c.mm. against an increase of 675 per c.mm. in the control. In the differential count the increase of polymorphs and the corresponding decrease in the percentage of the lymphocytes were not at all a distinguishing feature, as also there was no change in the percentage of other leucocytes, such as the large mononuclears, basophils and eosinophils. The Arneth index however, showed a tendency of shift to the right in the case of the animals subjected to the ultraviolet rays.

The infrared rays, likewise, had practically no effect on the differential count of the white blood cells although there was a slight tendency of shift to the right in all the exposed animals. The total count, however, was increased in two animals by 1250 per c.mm., whereas the last of the group had much less increase.

The platelets were enormously increased after both ultraviolet and infrared irradiation although the normals did not show any change whatsoever. Almost immediately after the first exposure to ultraviolet rays the platelets were increased by 200,000 per c.mm. and then there was a further increase of 10,000 per c.mm. in the course of the subsequent exposures. The increase after the infrared irradiation was on the other hand noted after several exposures.

The coagulation time was lowered by half a minute (from 2½ to 2 min) after ultraviolet exposures and by a quarter minute (2½ to 2¼ min) after infrared exposures.

All the above findings point to a definite stimulation of the hæmatopoietic system, especially regarding the percentage of hæmoglobin, total red and white cell count, platelet and reticulocyte counts. This is quite in keeping with Aitkin's[4] observation that "there is a definite stimulation of the hæmopoietic system. Practically in all cases there is an increase of red cells and also an increase of hæmoglobin and the latter may remain above the normal for a considerable time even after the treatment has ceased". But although the R.B.C., hæmoglobin, the polymorphs and platelets were found to respond by increase after a few exposures, there was no hurried production of these cells in the red bone marrow as was shown by a tendency of

shift to the right by Arneth index, as also a very slight increase in the percentage of the reticulocytes.

Regarding the leucocytic count, Tosi[5] observed leucocytosis immediately after irradiation, then leucopenia from half to one hour and later on another phase of leucocytosis after 6-24 hours. In this experiment blood was always taken immediately after the exposures, which revealed an increase in the leucocytic count in almost all the cases.

According to Aitkin[6], "Polymorphs are sometimes increased and sometimes diminished. The same is found with the lymphocytes. Some observers say that there is usually an increase in the polymorphs with a decrease in the lymphocytes while others say that the reverse is the case. But all observers would appear to agree that there is an increase in the number of eosinophiles. The number of platelets is also increased." As will be evident from the above tables, our observations are similar to those of the second school, as regards the relation of the polymorphs to the lymphocytes, that is, there is an increase in the polymorph count with a corresponding decrease in the lymphocytes and this finds corroboration from Clark[7] who found that although ultraviolet rays (shorter than 3000 A.U.) induce a greater increase of lymphocytes while the polymorphs remained constant, the near ultraviolet rays just short of the visible spectrum have a depressing effect on the lymphocytes and gave rise to an increase in neutrophils lasting for several hours.

Some observers have noticed a definite increase in the bactericidal power of the blood after ultraviolet exposures and Edinow[8] has shown that this was probably due to changes that develop with the circulation of blood through the irradiated area. The enormous increase of the polymorphs at the expense of the lymphocytes together with the total leucocytosis, probably contributes its quota for increasing the bactericidal power of the blood.

But regarding the eosinophils our findings are contrary to Aitkin's[9] as we could not notice any change in the percentage of these cells. On the other hand, the increase in the number of platelets as observed by us, is quite in accordance with the observation of Aitkin[10] and Clark[11].

The diminished coagulation time can be very well explained by the immense increase in the number of platelets although Aitkin[12] thinks that blood clots more rapidly after ultraviolet exposures, partly due to increased calcium content and partly due to augmentation of the number of platelets.

The increase in the percentage of the reticulocytes speaks of the formation of newer red blood corpuscles. Hobert[13] also found that after bleeding with loss of 50 p.c. of the red cells, regeneration was complete in 13-14 days after exposures to ultraviolet light.

In the infrared experiment, our observations were unluckily hampered by the sudden death of two experimental animals that developed pneumonia and died within a few hours in the middle of the experiment. Post-mortem examination revealed pneumonic condition of the two lungs in both the cases. This was probably due to the fact that after exposure to the infrared (heat) rays

which have a much greater penetrating power than the ultraviolet rays, the young animals could not stand the chill of the cold February nights and as such developed pneumonia. So unhappily the observations have to be restricted on the examination of blood of one experimental animal only that survived to the last but the effects as far as we noticed for about three weeks during which all the three eperimental animals were alive, were in many respects similar, though less in degree, to those due to ultraviolet rays, especially so far as percentage of hæmoglobin, total red and white blood cell counts, reticulocytic and platelet counts, Arneth index and coagulation time are concerned, although TROUPE[11] is of opinion that there is a difference in the effects produced in the blood, *e.g.*, infrared rays produce leucopenia whereas ultraviolet rays produce lymphocytosis.

SUMMARY

1. Neither ultraviolet light nor infrared rays have apparently any effect on the weight of the animals.

2. Ultraviolet irradiation has undoubtedly a stimulating influence upon the hæmatopoietic system but it does not cause any hurried production of the cellular elements. The number of the red blood cells with the corresponding amount of hæmoglobin and sometimes even more, is definitely increased. The increase in the leucocytic count is probably partly responsible for the increased bactericidal power observed by some observers. The blood clots rapidly owing to an increase in the number of platelets. There is some reticulocytic response too.

3. Infrared rays with their greater penetrability have effects on the blood similar but to a less degree than the ultraviolet rays.

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The Indian Journal of
Physiology and Allied Sciences
Vol. III, Nos. 1 & 2, 1949.

A NOTE ON THE PHARMACOLOGICAL ACTION OF COTARNINE PREPARED FROM INDIAN OPIUM ALKALOID

By

A. N. Bose and J. K. Ghosh

(From Bengal Immunity Research Laboratory, Baranagore)

Cotarnine hydrochloride (B.I.) prepared from Indian Narcotine was subjected to toxicity tests, and certain pharmacological investigations. The standard for reference was taken to be a pure sample of cotarnine salt obtained from E. Merck and Company, Germany.

Experimental

Toxicity Tests:

These were carried on mice and guineapigs, bred in our own Laboratory. The substance was injected intramuscularly in watery solution, the symptoms after injection were observed and the mortality noted upto 3 days. Both the standard and the test cotarnine solutions were used simultaneously on each day of the tests: Table I gives the result of the tests carried out on Mice.

TABLE 1

White mice weight 14 to 16 gms.

Drug	*Dose gm/kg*	*Mortality*	*Symptoms*
Standard Cotarnine Hydrochloride	0·1	0/8	Nil
	0·15	0/8	Shock, slow respiration but bigger excursion.
	0·17	5/12	Shock, slow respiration, Dyspnœa followed by death with respiratory failure or in convulsions.
	0·2	9/12	As above.
Cotarnine Hydrochloride (B.I.)	0·1	0/8	The sequence is same as standard with progressively increasing doses.
	0·15	0/8	
	0·17	6/12	
	0·20	9/12	

Toxicity in guineapigs:

It was found thatt 0·1 gm per kg was tolerated with no symptoms. Symptoms started at 0·15 gm per kg with dullness and ataxia followed by slow but deeper respiration leading to asphyxial convulsions and death in some of the animals (3/5). The certain lethal dose seemed about 0·2 gm per kg (5/5). There was no significant difference in the mortality figures between the test and standard solutions. Postmortem examination of the dead guineapigs which occurred in 1/2 to 2 hours, showed along with congestion of the Livers and Kidneys, congestion of right auricles which were found beating even one hour after death.

Action on isolated uterus:

Isolated virgin guineapig uterus was found to be definitely stimulated, though smaller in comparison with posterior Pituitary extract. The contractions were always relaxed by adrenalin.

Action on Blood pressure:

(*a*) In spinal cat, the blood pressure was somewhat raised (12 mm of Hg) with 1 mg per kg dose, but higher doses did not give proportional increase (Curve I).

(*b*) In chloralosed cat with intact vagus, the blood pressure with a small transient rise, was immediately lowered, followed by a rise to the original level; but, with subsequent repetition of the same dose (1 mg per kg) the amount of lowering tended to decrease (Curve II).

(*c*) In chloralosed cat after atropine:—The most peculiar reaction which occurred in both the experiments was the sudden stoppage of respiration after intravenous injection of Atropine (gr. 1/100) in chloralosed cats, which were already injected with cotarnine. The effect was so sudden that full atropinisation could not be accomplished. (Curve II). After stoppage of respiration, the heart was revived with artificial respiration but the initial level of B.P. was no more attained and the animal seemed to behave like a spinal cat. That is, adrenalin gave good responses, and cotarnine, instead of a fall, either gave no rise or at best a rise not more than 5 mm of Hg. (Curve III).

On Pulse rate:

Pulse rate was found to be unaffected at the beginning but after the B.P. level began to diminish after a series of injections, it began to increase. The output of the heart, as judged by the excursions of the pulse was not found to be much affected.

On Respiration:

In one experiment. the excursions of the respiratory movement was found to be definitely stimulated after each injection of 0·2 c.c. (1 mg/kg), but the rate remained fairly steady (Curve II). In another experiment no change whatsoever in excursion or rate was found.

On artificial adrenalin hypertension:

Hypertension caused by adrenalin infusion in chloralosed cats, was immediately lowered by the injection of cotarnine (1 mg/kg) inspite of the continued administration of adrenalin (1/100,000). But, on continual infusion of cotarnine, adrenalin did not bring about any appreciable rise in blood pressure. (Curves IV & V).

Discussion

From the toxicity experiment it is apparent that the guineapigs and mice, bred under Indian conditions, tolerated more of cotarnine than what is given by Sollmann (1936) who described the lethal dose for guineapigs to be 0·1 gm per kg. Whether the difference is due to higher resistance of our animals has got to be investigated.

From the pharmocological experiments, we find that cotarnine in chloralosed cats with intact central nervous system produces a definite fall in blood pressure but in a spinal cat, it produces a definite though smaller rise. This depressor effect is so powerful that even in artificial adrenalin hypertension, the effect is marked. Moreover, it is found that the higher the hypertension, the better the depressor effect. On the contrary, Adrenalin cannot produce a pressor effect on cats, having infusion of cotarnine. This phenomenon shows some sort of antagonism between adrenalin and cotarnine. The depressor action of cotarnine can not be explained by the depression of the heart, as no alteration of the pulse could be observed. The question, however, arises as to why the blood pressure is raised in spinal cats. This can possibly be explained by the direct sttimulation of the arteriole musculature or nerve endings in the system, though direct action on arterioles could not be demonstrated in perfusion experiment (Sollmann, 1936). This idea gains support by its direct stimulatory action on isolated uterine musculature, and intestines (Offergeld, 1915). The failure of respiration after atropine, is the most difficult factor to explain. Whether this is due to over-sensitisation of the para-sympathetic system by doses of cotarnine which reacted to the sudden paralysis of vagal nerve-endings by atropine, or due to stimulation of the afferent sensory fibres of the vagus leading to complete inhibition of respiration, has got to be fully investigated.

Work along these lines are being taken up for further investigation.

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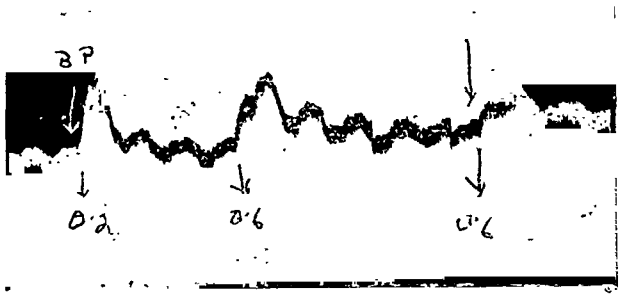
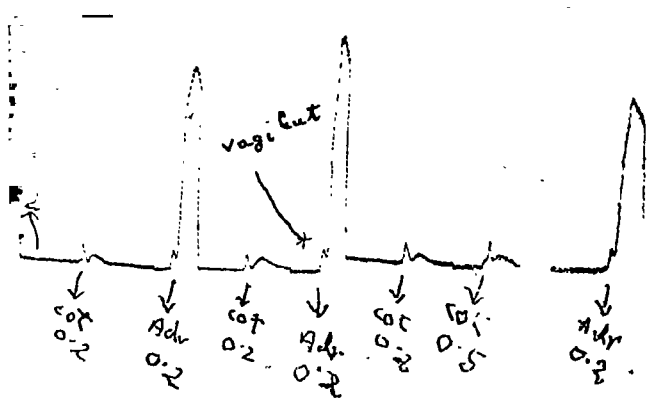
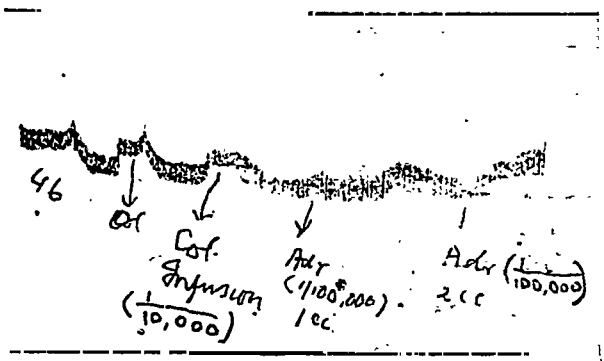


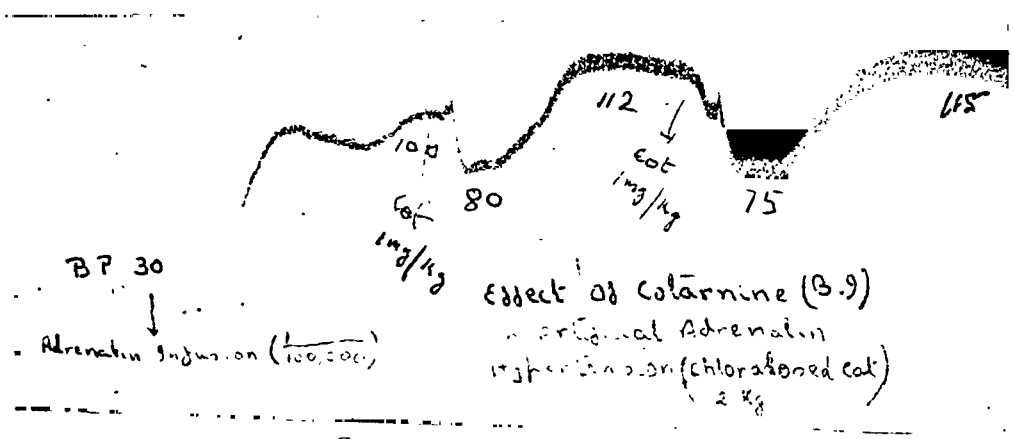
CURVE I. Spinal Cat



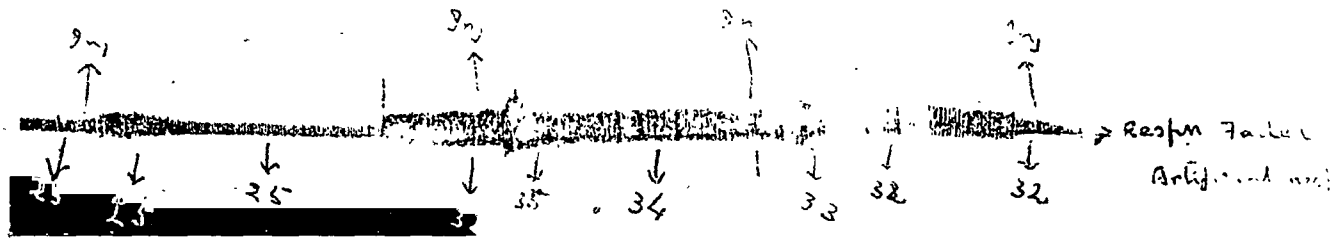
CURVE III. Chloralosed Cat after atropine



CURVE V. Chloralosed Cat showing effect of Adrenalin after cotarnine infusion



CURVE IV. Chloralosed Cat



CURVE II. Chloralosed Cat—showing the effect of cotarnine (B.I.) on B.P. and respiration after each injection of 0·2 c.c. (1 mg/kg) Portions of respiration and blood pressure curves are corresponding, the numbers in the upper curve indicate rate of respiration per minute, and the number in lower curve, the blood pressure in mm of Hg.

PRELIMINARY OBSERVATIONS ON THE ASSAY OF LIVER EXTRACTS ON SPLENECTOMISED RABBITS*

By

A. N. Bose.

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Various attempts have been made to standardise Liver Extract preparations by biological methods in the laboratory. But none has uptill now been accepted as a uniformly suitable method. Production of pernicious type of anœmia being difficult in animals, recent trends in assay have undergone a change from the study of artificially produced diseased animals to the study of reticulocyte response in normal animals. Thereby, methods have been developed with pigeons,[1] guinea-pigs,[2,3,4] and rabbits.[5] Recently, Jacobson and Williams[5] have reported that splenectomised animals show a high reticulocyte response to injection of potent liver extracts. Inorder to see whether this method of assay in splenectomised rabbits would serve as a routine laboratory technique for judging the potency of different forms of fractionated and crude liver extracts, a number of experiments have been carried out in this laboratory, the results of which are incorporated in the present note.

Experimental

Three rabbits, all male, weighing between 1·5 and 2 Kg. were splenectomised under ether anæsthesia. Atropine sulph. 1/120 gr. was injected in each animal prior to anæsthesia. The abdomen was opened by an oblique incision about 1½ inch lateral to the rectus abdominis muscle and starting downwards from the angle of the ribs. The spleen was taken out after ligaturing the stock with catgut, muscles being replaced and stitched with catgut; the skin was sutured with silk worm gut, and the parts bandaged with necessary antiseptic precautions. The next day the animal was found to be normal. Stitches were removed on the 8th day.

Normal daily reticulocyte counts were done (Bose *et al*[6]) after 10 days' period of rest after operation. The counts remain high during the first week, but gradually come down in 10 to 12 days. No animal was taken for study of liver extracts till the daily count attained a normal which varied between 0·7 to 1·3%. After 7 days' observation

of normal reticulocyte counts, intramuscular injection of the test liver extract was made, the dosage being, per kg. body weight, 1 c.c. of a Daily reticulocyte counts were done for 7 days after the injection. Responses were recorded as positive if the reticulocyte count increased by 100 per cent or more of the normal maximal level, minimum figure acceptable being at least 2 per cent. After a course of observation, the animal was allowed rest for 7 days or, till the reticulocyte regained its normal level. A second course of observations with another sample was then made again. Six samples were taken for observing the reticulocyte response in splenectomised rabbits. These were Neo-hepatex (Evans), Lily's Liver Extract (crude), T.C.F. Liver Extract, Bi-Liverex, whole crude liver extract No. W_3 and Casein hydrolysate containing equivalent amount of nitrogen per c.c. as that in Bi-Liverex.

* Read at a meeting of the Section of Physiology, Indian Science Congress Session, Allahabad, 1949.

The Table summarises the results of these experiments.

TABLE

Showing the reticulocyte response of splenectomised rabbits, after injection of various Liver preparations and Casein hydrolysate.

Splenectomised Rabbit No. I			*Splenectomised Rabbit No. II*			*Splenectomised Rabbit No. III*		
Days of observations	Reticulocyte percentage	Response	Days of observations	Reticulocyte percentage	Response	Days of observations	Reticulocyte percentage	Response
1	1·0		1	0·7		1	1·4	
2	1·3		2	0·8		2	1·3	
3	1·0		3	1·0		3	1·4	
4	1·1		4	1·3		4	1·2	
5	—		5	1·2		5	1·6	
6	0·7		6	1·2		6	1·1	
7	Injected with 1 c.c. per kg of 1/100 dilution of Neo-Hepatax.			Injected with 1 c.c. per kg of 1/100 dilution of T.C.F. Liver Extract.			Injected 1 c.c. per kg of 1/125 dilution of Bi-Liver Batch 51047.	
8	1·1					7	1·8	
9	3·0	Positive	7	1·5		8	5·0	
10	1·8		8	2·6		9	2·8	Positive
11	1·8		9	2·1	Positive	10	7·8	
12	0·9		10	2·0		11	2·7	
13	1·0		12	2·0		12	—	
			14	1·5				
Rest for 10 days			Rest for 10 days			Rest for 10 days		

Splenectomised Rabbit No. I			*Splenectomised rabbit No. II*			*Splenectomised rabbit No. III*		
Days of observations	Reticulocyto percentage	Response	Days of observations	Reticulocyto percentage	Response	Days of observations	Reticulocyto percentage	Response
1	Scanty		1	0·6		1	0·6	
2	"		2	1·5		3	0·6	
3	1·0		3	—		4	0·9	
4	0·7		4	0·6		5	0·8	
5	0·7		5	0·6				
	Injected with 1 c.c. per kg of 1/100 dilution of Liver extract W_2 (whole crude)							
6	—		6	0·5		6	0·8	
				Injected with 1 c.c. per kg. of Casein Hydrolysate.			Injected with 1 c.c. per kg. of Casein hydrolysate	
7	0·8		7	0·8		7	—	
8	1·8	Negative	8	0·8		8	1·5	
9	1·4		9	Scanty	Negative	9	1·9	Negative
10	1·2		10	0·3		10	1·2	
Rest for 10 days			12	0·3		12	0·9	
1	0·5							
2	0·7							
5	1·0							
7	1·0							
	Injected with 1 c.c. per kg of Lily's Liver Extract (crude)							
8	1·5							
9	2·4							
10	2·4							
11	2·8							
12	0·5							
13	1·5	Positive						

Discussion

From the Table, it is evident that splenectomised rabbits give rise to a higher reticulocytosis in response to injections of potent liver extracts than those given by guineapigs,[2,4]. That non-specific substances do not bring about such increased response can be seen from the results of the reticulocyte count done after injection of casein hydrolysate and an inactive Liver Extract preparation W_3. Unlike guinapigs (cf),[2] these rabbits are however susceptible to giving response to repeated injections of potent preparations. It is therefore possible to take reading of different doses or preparations on a single animal. The observation so far recorded here as well as those by Jacobson and Williams (*loc. cit.*) however show that the response is of a qualitative nature. Further work is being carried out to find out whether the same animal would give a quantitative response with a graded dosage of the same Liver Extract.

Conclusion

Attempts have been made to find out a suitable method of assay of liver Extract in splenectomised rabbits. It is being found that a definite qualitative idea regarding the potency of liver extracts can be had from the reticulocyte count of splenectomised rabbits after injection.

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MEETINGS OF THE PHYSIOLOGICAL SOCIETY OF INDIA

An ordinary meeting of the Physiological Society of India was held in the Department of Physiology, Presidency College, Calcutta, on the 10th May, 1949.

Prof. B. B. Sarker presided. Dr. S. Banerjee, Professor of Physiology, Presidency College, Calcutta, presented the following communications:

(*a*) Thyroid Gland in Scurvey by S. Banerjee and N. C. Ghosh.

(*b*) Estimation of Total Carbohydrates in food stuffs by S. Banerjee and N. Nundy.

In the first paper, the histopathological changes in the thyroid gland of antiscorbutic guinea pigs were demonstrated by slides and also by epidiascope. The technique of producing scurvey artificially in guinea pigs was described and the possible role of vitamin C on thyroid gland etc. were discussed.

A method for the estimation of total carbohydrates in food stuffs was then presented. This paper is being published in this issue of the Journal.

The second ordinary meeting of the Physiological Society of India was held on the 7th June 1949 at the Lecture Theatre of the Department of Physiology and Biochemistry in the Calcutta Medical College.

Dr. M. C. Nath of Nagpur University read a paper on 'Experimental Diabetes by intermediary fat metabolites and the comparative effects of insulin and amellin'.

Prolonged injection of sodium aceto-acetate has been found to develop hyperglycemia in the normal rabbits through an initial hypoglycemic stage and to show typical diabetic glucose tolerance curve, increased glycogenolysis and accumulation of lactic acid in the blood.

Effects of injection of 'insulin' and 'amellin' have been studied on such induced diabetic animals.

Animals receiving amellin in addition to the Ketone bodies for about one month showed absolutely normal glucose tolerance curve and far better results than those receiving 'insulin' and Ketone bodies.

A symposium on the need for study and research in higher Physiology in India was held by the Physiology students of the Union in collaboration with the Physiological Society of India on 26th Feb-

ruary, 1949 at the University College of Science. The following took part on the subjects mentioned below:

(1) Dr. B. B. Sarker—'Physiology in India'.
(2) Dr. B. Mukerji—'Scope of Applied Physiology'.
(3) Dr. S. Banerjee—'Role of Biochemistry in National Development'.
(4) Mr. N. M. Basu—'Need for fundamental researches'.
(5) Mr. S. R. Maitra—'Difficulties of the students of Physiology'.

The detailed paper and Discussions will be printed in the next issue of the Journal.

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The Indian Journal of Physiology and Allied Sciences

Vol. III JANUARY, JULY, 1949 Nos. 1 & 2

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Printed and published by
NIBARAN CHANDRA DAS,
Prabasi Press, Calcutta.

THE INDIAN JOURNAL OF PHYSIOLOGY AND ALLIED SCIENCES

October 1949

Published by
THE PHYSIOLOGICAL SOCIETY OF INDIA,
CALCUTTA

Price Rs. 16 Per volume

"In a world struggling for unity, border lines between the different spheres of human thought become unessential."

Thomas Mann

"It is the avowed purpose of scientific thought to reduce the number of mysteries, and its success has been marvelous."

J. H. Robinson

"About the year 1857 Johannes Muller broke down mentally trying to keep abreast of the literture on physiology."

E. J K. Menge

The Indian Journal of
Physiological and Allied Sciences
Vol. III, No. 3. 1949.

ON THE NATURE AND OCCURRENCE OF THE PANCREAS IN SOME INDIAN TELEOSTEAN FISHES.

By
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Introduction

Macallum (1884) for the first time reported the presence of pancreas embedded in the liver around the interlobular veins in a teleost, *Amiurus catus*. He later (1896) extended his observations in some Ganoid fishes and reported that this gland may be completely or partly diffused in those fishes and as such lies scattered more or less over the viceral cavity. It is also found associated with the portal vessels within the substance of the liver. Similar observations were recorded by Stohr (1893) Goeppert (1893), Laguesse (1894, 1895), Gulland (1898), Hill (1926), *et al* in a large number of teleostean fishes. Smallwood and Derrickson (1934) described the pancreas in *Cyprinus carpio* as a complex diffused mass enclosed within the liver. He further pointed out that the pancreas originates from certain undifferentiated cells which proliferate from the 'right liver duct' and extend dorsally over intestine. Sarbahi (1939) reported that in *Labeo rohita* pancreas is apparently absent but is really present as a diffused mass scattered more or less all over the visceral cavity and also within the substance of the liver'. According

to him the pancreas is firstly found as a 'parivascular tissue surrounding the finer branches of the blood vessels ramifying the adipose tissue attached to the coil of the intestine' ; secondly 'a considerable portion of the pancreas is imbedded within the substance of the liver accompanying the ramifications of the portal vessels and the hepatic artery'. He further recorded that the pancreatic tissue is found 'imbedded in the substance of the spleen around its smaller bloodvessels'.

After a review of the above works it seems evident that the pancreas is present in both the teleostean and ganoid fishes and further it is closely associated with the liver. However, the evidences regarding the exact location of the pancreas and its relationship with other associated organs seem to be meagre. Moreover, there is a great paucity of informations regarding the histological details of the pancreas in fishes and also regarding the nature of the pancreatic duct. The present investigation was, therefore, undertaken in order to provide additional data on the point.

Material and methods.

The following eight species of teleostean fishes were involved in the present investigation : (1) *Labeo rohita* (Ham.), (2) *Cirrhina mrigala* (Ham.), (3) *Cirrhina reba* (Ham.), (4) *Barbus stigma* (Cuv and Val.), (5) *Silondia silondia* Ham., (6) *Mugil parsia* (Ham.), (7) *Lates calcarifer* (Bloch.), and (8) Ophicephalus punctatus (Bloch.). The livers were dissected out, washed in normal saline and were finally fixed either in Brasil Duboscq's modification of Bouin's fluid for histological study or in Zenker-formol-acetic mixture. Serial sections, 6-10 micra thick, were prepared by the paraffin method and were stained with Ehrlich's haematoxylin followed by alcoholic eosin or with Mann's methylene blue and eosin. Sections of the mesentery, supra intestinal adipose tissue and the spleen were also prepared by the above procedure in order to find out the presence of pancreatic tissue in these structures.

Observations.

Since the nature and distribution of the pancreatic tissue appear to be more or less identical in the eight species of the fishes involved in the present study, we have attempted to give a generalized description based on careful examination of serial sections through the liver of the fishes.

The pancreas, in all the fishes studied, is sparingly distributed as patches over the surface of the hepatic lobes (Figs. 1, A, & C.*p.*) connected by thin strands of pancreatic tissue lying within delicate stroma (Fig, 1. C, *C.s.*). The superficial pancreatic patches and their anastomoses practically surround the whole of the middle region of the hepatic lobe. Each superficial pancreatic patch produces branched racemose structure which penetrates into the substance of the liver in a manner analogous to a plant parasitized by fungus possessing mycellia. The liver can be compared with the host plant, the penetrating branched pancreatic tissue with mycellia and each superficial patch can be analogised with the body of the fungus.

An examination of both transverse and longitudinal sections reveals that the pancreatic patches remaining outside the hepatic lobes contain a few pancreatic patches only (Fig. 1 C, *P*) and are incipiently glandular in nature. Each strand connecting the patches contains a very narrow channel in its middle (Fig. 1 C, *Ch*) which probably serves as a passage for transportation of pancreatic products from one patch to the other. The pancreatic tissue which penetrates inside the liver is completely glandular (Figs. 1 A and C, P. G.) and remains separated from the cells of the hepatic lobules by interlobular connective tissue extended from the capsule of Glisson (Figs. 1 A and B, B. m.). The pancreas does not possess a distinct connective tissue capsule but is covered in certain regions by a thin layer of loose connective tissue (Fig. 1 B, P. m.).

In transverse sections the serous alveoli are found more frequently towards the periphery and to a lesser degree towards the centre. The acinar cells lining the alveoli can easily be distinguished from the large polyhedral hepatic cells having

intense cytoplasmic vesiculation as well as from the stellate Kupffer cells by their structure and staining reactions. The acinar cells are more or less columnar in shape and appear dark bluish violet in haematoxylin and eosin stained sections, whereas the cells of the liver look pinkish red in similarly stained preparations. Moreover, the acinar cells of the pancreas are partially filled with granules. A few spindle-shaped centro-acinar cells are also occasionally found close to the acinar cells.

Islets of Langerhans (Fig, 1 B, *I. s.c.* and Plates. 1 and 5, *I.s.c.)* appear as small cells remaining in close association with the acinar cells. Some islets are also found scattered in the hepatic mass (Fig. 1 A. *Is. C.*) The islet cells take up faint stain in haematoxylin and eosin preparations, and, therefore, they stand in sharp contrast with the deeply stained acinar cells, The islets of Langerhans are rarely found in the pancreatic patches distributed over the surface of the liver. These are often found to be supplied with large and irregular capillaries.

The pancreas possesses no external duct of its own. Indeed, small ductules rising from the branched racemose glands never join to form a single pancreatic duct. The ductules are found scattered in the substance of the liver and each of them, after a short course, joins with the branch of an inter-lobular bile duct (Plate, 2. P. D. and B. D.) to produce a common duct for both the liver and the pancreas. These ducts unite and form larger and larger ducts and finally join to form the hepatic ducts. Each pancreatic ductule is lined internally by simple columnar cells and outside there is a connective tissue serosa. The branch of the inter-lobular bile duct can be distinguished easily from the pancreatic ductules by its lining of cuticular membrane and the absence of single layered columnar epithelium. Moreover, the microscopical appearance of the secretions present in the lumen of the ducts are quite different. This can be appreciated by a examination of the photomicrograph (Plate, 2, B. S. and P. S.) which depicts a condition just before the mixing up of the pancreatic juice with the bile.

A careful examination of the sections of the mesentery, supra-intestinal adipose tissue and the spleen failed to show the

Explanation of Plates.

Plate. 1. Transverse section showing pancreatic tissue entering within the substance of the liver in *Lates calcarifer* (x 60).

Plate. 2. Transverse section showing the relationship between pancreatic duct and the bile duct within the substance of the liver in *Labeo rohita* (note the nature of the secretions presenting in the lumen of both the ducts) (x120).

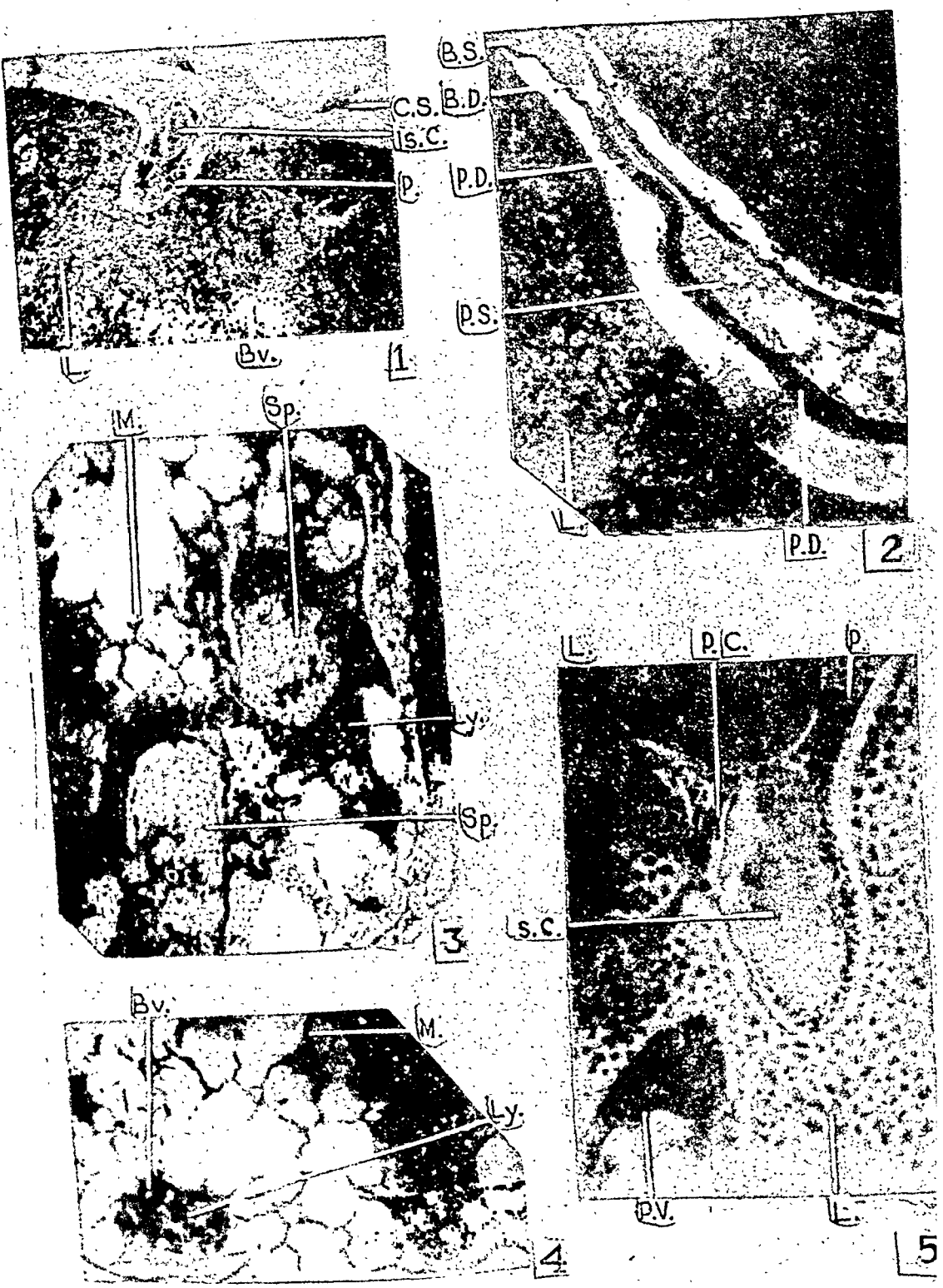
Plate. 3. Transverse section of the Spleen showing Lymphatic tissue encircling the portion of the splenic tissue in *Labeo rohita*. Portion of Mesentery entering within the Spleen along with the Lymphatic tissue is also seen (x60).

Plate. 4. Section of Mesentery of *Labeo rohita* showing the presence of Lymphatic tissue (x 60).

Plate. 5. Transverse section of the liver of *Lates calcarifer* showing the structure of pancreas practically surrounding an islet of Langerhans; portal vein is seen off from the pancreas (x60).

Abbreviations used in Plates and Figure.

B. D.—Branch of the interlobular bile duct ;—B. S.—Bile secretion ; B. V.—Blood vessel ; C. S.—Connective tissue stroma ; Is. c.—Islet of Langerhans ; L.- Hepatic portion of the compound gland ; Ly —,Lymphatic tissue ; M.—Mesentery ; P.—Pancreas ; P. C.—Pancreatic cell (acinar type) ; P. D.—Pancreatic duct ; P. S.—Pancreatic secretion ; P. V.—Portal vein ; Sp.-Spleen.



Plates 1—5

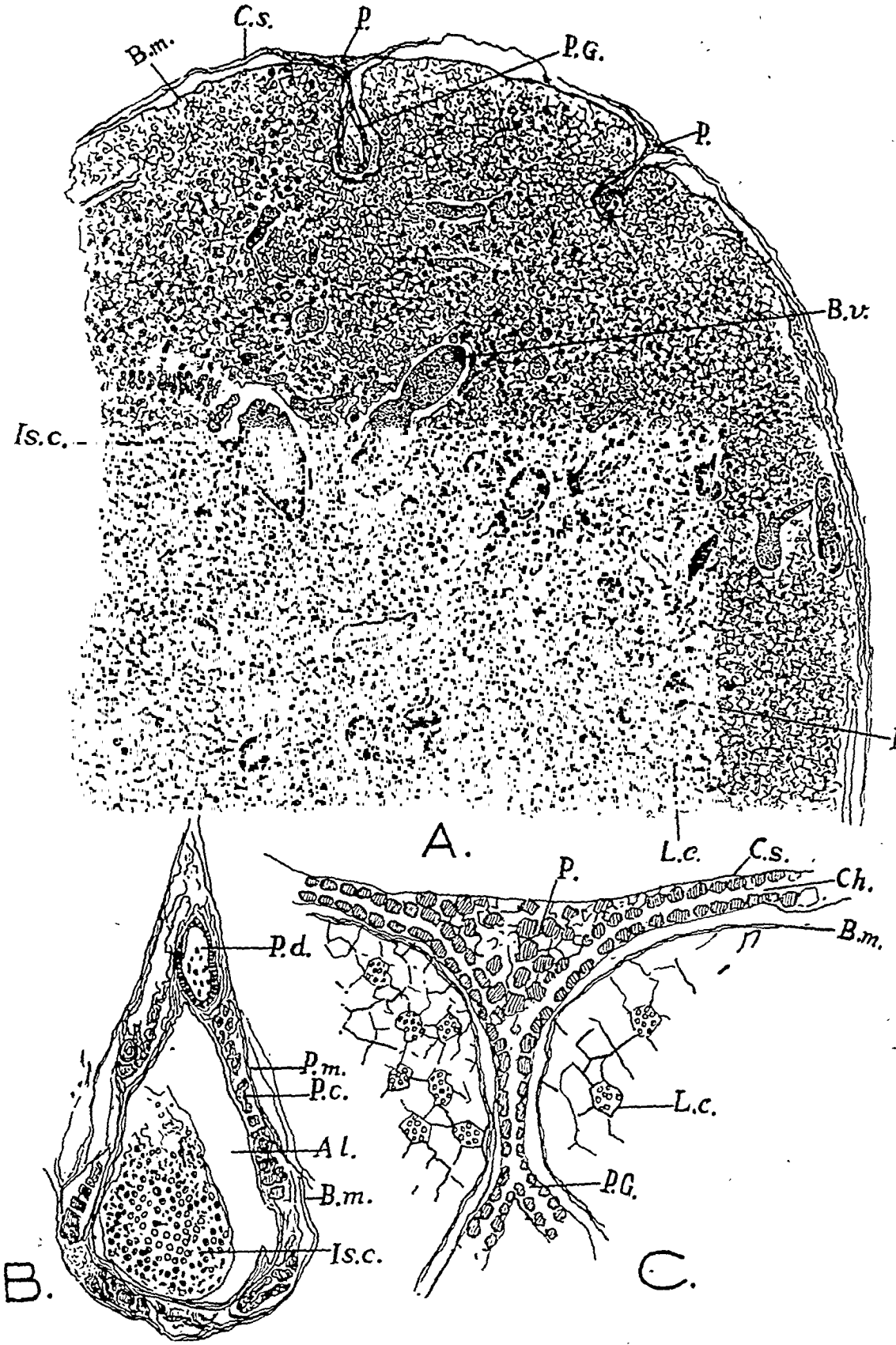


Fig. 1

presence of any typical pancreatic tissue as suggested by Sarbahi (1939). However a particular type of tissue made up of lymphoid cells has been observed in those structures. The histological structure of this tissue (Plates, 3 and 4. Ly) appear to be quite distinct from that of the pancreas found in the liver.

Discussions.

Our results seem to be at variance with those of Sarbahi (1939) who reported the presence of diffused pancreatic tissue in the mesentery adipose tissue and spleen of *Labeo rohita*. However, our observations on the same species definitely indicate that the diffused tissue identified as pancreas by Sarbahi is actually lymphoid in nature.

Our present knowledge regarding the occurrence of the islets of Langerhans and their relationship with the pancreatic tissue in the teleostean fishes is from the work of Rennie (1902-03 and 1905) and Sarbahi (1939). According to these authors, in some cases the islets lie alongside the pancreatic tissue and remain loosely attached to its acini, while in other cases the pancreatic tissue penetrates the tissue of the islets. Sarbahi (1939) further opines that the second condition is due to the diffused nature of the pancreas, Since no islet tissue has been observed in the diffused extra-hepatic pancreas, either in the visceral adipose tissue or in the spleen, he is also of the opinion that this tissue is confined to the intra-hepatic portion of the pancreas. Carleton (1938) states that the islet tissue in teleostean fishes forms a separate organ but remains attached to the rest of the pancreas. However, we never found such cases where the islets were infiltrated by the pancreatic tissue or they formed separate organ by themselves.

Sarbahi (1939) is of opinion that the pancreatic duct opens separately into the gut in close association with the hepatic duct but we have observed that the pancreas does not possess any external duct of its own. The pancreatic ductules arising from the racemose structures open into the bile ductules within the substance of the hepatic mass and, therefore, the so-called bile

duct may actually be regarded as the hepato-pancreatic duct since it carries the secretions of both the liver and the pancreas.

The present study further reveals that the liver is not a homogeneous mass in the species under review and is quite inseparable from the pancreas which forms a complex structure by producing pancreatic alveoli, islets of Langerhans and the pancreatic ductules within the substance of the liver itself. We, therefore, conclude that the liver in these fishes is in the nature of a hepato-pancreas.

The authors are indebted to Dr. S. P. Ray Chaudhuri, Zoology Department, Calcutta University for his help in the preparation of the manuscript for the press. Sincere thanks are due to Mandar Studio, 209 Cornwallis street for preparing the photomicrographs.

Summary.

1. The nature of the pancreas in eight species of Indian teleostean fishes (*Labeo rohita*, *Cirrhina mrigala*, *Cirrhina reba*, *Barbus stigma*, *Silondia silondia*, *Mugil parsia*, *Lates calcarifer* and *Ophicephalus punctatus*) has been studied.
2. The pancreas in all the species studied is present as thin patches over the substance of the liver and also penetrating into its substance.
3. The pancreas possesses no external duct of its own. Small ductules from the pancreas join similar ductules of the liver to form common hepato-pancreatic duct.
4. The mesentery, visceral adipose tissue and the spleen do not show the presence of pancreatic tissue as suggested by Sarbahi (1939). These structures, on the other hand, appear to be infiltrated by a kind of lymphoid tissue.
5. In view of the close association between the pancreas and the liver, it appears that the latter is in the nature of a hepato-pancreas.

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Tha Indian Journal of
Physiology and Allied Sciences
Vol III, No. 3. 1949.

A STUDY OF BROMOBENZENE DETOXICATION IN RATS BY RADIOACTIVE METHIONINE

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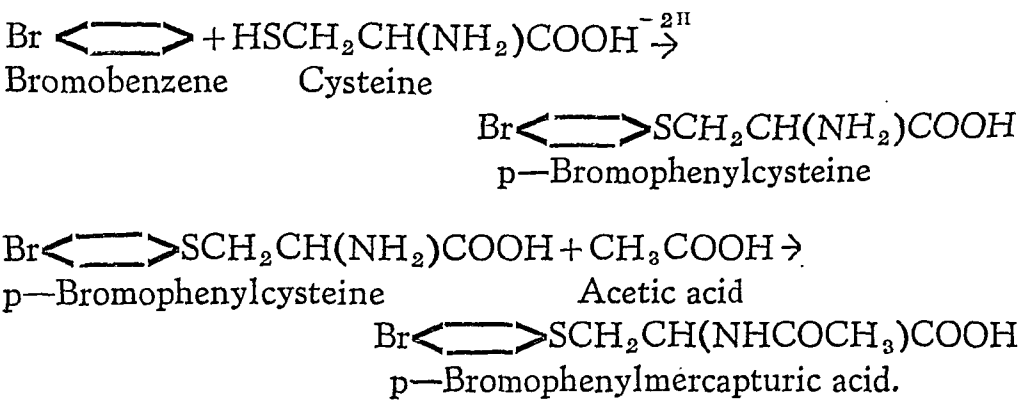
Monohalogen derivatives of benzene such as bromobenzene when fed to an animal, especially a mammal are usually detoxicated by conjugation with cysteine, and subsequent acetylation of the products results in the formation of mercapturic acid.



When cysteine and methionine are excluded from the diet, halogen derivatives of benzene are oxidised to phenols and conjugated with sulphates. Hydroxy-compounds are readily conjugated with inorganic sulphates and the resulting ethereal sulphates are excreted in urine. Feeding of bromobenzene in

normal animals increases the urinary excretion of neutral sulphur and decreases the growth rate, which may be corrected by inclusion in the diet of glutathione, cysteine or methionine (Stekol 1941, 1943 ; White & Jackson 1935 ; Zbarsky & Young, 1943). Cysteine required for this detoxication is derived from cysteine or methionine. In this work an attempt has been made to find out how much of the methionine introduced in the body will be utilised for the formation of cysteine to help the detoxication of bromobenzene.

Experimental

Radioactive methionine with S^{34} in a dose of about 4,000-5,000 counts/minute was used. Rats of Long-Evans strain were divided into 2 groups, the experimental group was fed 0.15 ml. of bromobenzene mixed with 0.35 ml. of cotton seed oil, the control group being fed 0.50 ml. of cottonseed oil only. One hour after this all the rats were fed 1 ml. of methionine solution and were put in metabolism cages with arrangements for the collection of urine. After 24 hours the rats were anaesthetised and blood was collected by cardiac puncture in oxalated tubes. Then these animals were sacrificed and the organs collected. Liver, kidneys, intestinal mucosa and thigh muscles were homogenised and treated with 10% trichlor-acetic acid. Blood plasma was similarly treated with trichlor-acetic acid.

Method

Urine collected during 24 hours was made up to 50 ml. with distilled water. For the estimation of Inorganic Sulphate 10 ml. of this diluted urine was mixed with 1 ml. of concentrated. hydrochloric acid, evaporated on steam-bath to dryness. The black residue was taken in water and filtered. 10 ml. of 0. 05 N sulphuric acid was added to the filtrate, 2 ml. of 2 N hydrochloric acid added, heated and then precipitated with 10% barium chloride solution. The precipitate was collected on a standard size filter paper, washed with 50% acetone and dried. This was later counted. For the estimation of Total Sulphur of urine, 10 ml. of

this diluted urine was mixed with 20 ml. of Pirie reagent (9 volumes of concentrated nitric acid and 3 volumes of perchloric acid, the mixture then added to 4 volumes of perchloric acid saturated with copper nitrate) and taken in a Kjeldahl flask with some glass beads and heated on a Bunsen burner for 24 hours. This was then further treated as in the case of similar tissues to be described. Homogenized tissues and blood plasma were precipitated with 10% trichloracetic acid and kept in a refrigerator overnight. The next day, they were centrifuged and washed with 5% trichloracetic acid three times and finally the samples were digested with 20 ml. of Pirie reagent as in the case of urine collected during 24 hrs for total sulphur. These were then evaporated to dryness. The residue was cooked with 2 ml of 6 N hydrochloric acid, and then transferred to Erlenmeyer flasks with 50 ml of water, 20 ml of benzidine hydrochloride (benzidine dihydrochloride 80 gm, 2 N hydrochloric acid 400 ml and water added to 2,000 ml) was then added and the mixture was kept overnight in a refrigerator. The next day this was filtered through a little filter paper after adding 4 ml of a saturated solution of filter-cell and the residue was transferred back to the Erlenmeyer flask after washing with 50% acetone. After adding 50 ml of water to it, it was heated to boiling, 4 drops of phenol red solution being added as indicator and then titrated with standard 0.05 N caustic soda to light salmon. An excess of caustic soda solution was now added. It was then cooled and filtered into a beaker. 0.05 N sulphuric acid was then added to it up to 10 ml equivalent (e. g, if titration with 0.05 N caustic soda gave a value of 3 ml, then 7 ml of 0.05 N sulphuric acid only was to be added), after adding 2 ml of 2 N hydrochloric acid, it was heated and precipitated with 20 ml of 10% barium chloride solution. The precipitate was collected on standard size filter paper, washed with 50% acetone and dried. The original methionine solution was diluted 200 times and aliquots treated like homogenized tissues and this served as the standard. Liver and muscle samples were run in quadruplicates and urine in duplicates. These were all counted in a Geiger-Muller counter with a thin window to allow weak beta-radiations from the radioactive sulphur

to act. From these counts standard specific activity (SSA) was calculated as follows :

$$SSA = \frac{\text{Weight of rat in kg} \times \text{Count of sample} \times 100}{\text{Titration value of sample} \times \text{Count of standard} \times \text{Dilution of standard} \times \text{Molarity of standard alkali}}$$

Results

These are summarised in tables 1 and 2. Table 1, shows a marked increase in radio-activity in total urine sulphur after feeding bromobenzene, while there is a decreased count in inorganic sulphate of urine. Thus while normal subjects excrete about 17% total sulphur and 4.5% inorganic sulphate after feeding radioactive methionine, after bromobenzene treatment total sulphur excretion rises to 25% and inorganic sulphate falls to 3. 9%. Table 2 shows an increase in count in kidney and liver of the experimental animals, with a decrease in intestinal mucosa, muscle and blood plasma.

TABLE 1.

Distribution of radio-sulphur in total sulphur and inorganic sulphate fractions of urine expressed as Activity %.

Treatment	Number of rats	Total S^*	Inorganic S^*O_4	Av. weight of rats	Total counts minute fed
Control	2	16.2	4.52	211 gm.	
Expt 1					5,500
Fed Bromobenzene	4	24.5	3.98	253 gm.	
Control	2	17.8	4.70	210 gm.	
Expt 2					3,350
Fed Bromobenzene	4	25.8	3.84	224 gm.	

Figures of total S^* and inorganic S^*O_4 are calculated as follows :

$$\text{Activity\%} = \frac{\text{count in urine} \times \text{Dilution factor} \times 100}{\text{count in standard} \times \text{dilution factor}}$$

S^* Stands for radioactive S^{35}.

TABLE 2.

Distribution of radio-sulphur in tissue proteins expressed as standard specific activity.

Treatment	Tissue				
	Kidney	Intestinal Mucosa	Blood Plasma	Liver	Muscle
Control Expt 1	2.84	3.09	2.51	1.58	0.249
Fed Bromobenzene	3.03	2.82	2.21	2.05	0.225
Control Expt 11	3.12	3.86	2.75	2.11	0.307
Fed Bromobenzene	3.43	3.64	1.84	2.31	0.285

Discussion

Sulphur of methionine is partly oxidised and excreted as inorganic sulphate in urine although a greater part is excreted in other fractions of urine sulphur. On feeding bromobenzene, in the presence of a ready supply of cysteine, cysteine combines with bromobenzene to form p-bromophenyl-cysteine which is excreted as ethereal sulphate. If under these circumstances the direct supply of cysteine be deficient, then methionine is gradually converted more and more into cysteine and is less oxidised directly, so that the inorganic sulphate excretion in urine falls, accompanied by a greater rise in the excretion of other sulphur-fractions.

The detoxication of bromobenzene occurs mostly in liver by combination with cysteine and the latter is also formed from methionine in this organ. The experiment is so arranged that bromobenzene first reaches the liver and later on methionine also arrives there. As soon as bromobenzene reaches the liver, liver proteins are decomposed, and cysteine and methionine obtained thereby are utilised for the detoxication of bromo-benzene. As soon as methonine arrives on this scene, it is immediately utilised partly for the detoxication of any remaining bromobenzene and partly for the re-synthesis of liver-proteins. But a small amount of methionine may leak into the systemic circulation. This may be at once incorporated into such parts

of the body where synthetic processes are more prominent. It is thus evident that radio-aciye Sulphur of methionine which is not used for detoxication, is expected to be present in the largest amount in liver and to a very small extent in certain parts of the body, The experimental results confirm this expectation. (Table 2)

During excretion through the kidney, bromobenzene-fed rats show a higher concentration of radio-sulphur in the kidney proteins. This incorporation of methionine into tissue proteins is retarded where there is an extra demand for conversion into cysteine.

Summary

1. Methionine with radio-sulphur was fed to rats some of which were also given bromobenzene.

2. There is a decreased excretion of radio-sulphur in organic sulphate and an increased excretion in total sulphur of urine in experimental animals.

3. There is an increased count of radio-sulphur in liver and kidney proteins, but decreased count in proteins of blood plasma, muscle and intestinal mucosa of the experimental group.

4. Incorporation of methionine into tissue proteins is retarded when there is an extra demand for its conversion into cysteine.

Acknowledgement

I am grateful to Dr. Harold Tarver of the Division of Biochemistry, University of California, Berkeley, U. S. A for the supply of radioactive methionine and helpful advice throughout this work.

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The Indian Journal of
Physiology and Allied Sciences
Vol. III. No. 3. 1949.

A RAPID CHEMICAL METHOD OF ESTIMATION OF THIAMINE IN FOOD STUFFS.

By

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SACHCHIDANANDA BANERJEE

(From the Department of Physiology, Presidency College, Calcutta)

Thiamine occurs in natural foods and other biological materials both in free and in combined forms. (Pyke, 1940; Ahmad et al, 1948) and the relative amounts of these forms vary considerably in different sources (Ahmad et al, 1948). Chemical analysis can be carried out more rapidly and economically and are more applicable to routine determinations than most of the other methods. Two principal types of thiamine assay have been used. (i) Determination of *thiochrome* produced by the oxidation of thiamine by alkaline ferricyanide and (ii) measurement of intensity of the color produced by coupling an amine such as para-amino acetophenone with thiamine. (Melnick and Field, 1939; Prebluda and Mc Collum, 1939). Of the several methods of estimation of thiamine in biological materials by the measurement of fluorescence which have been used during the last quarter of the century, the method of Jemsen (1936) has been used extensively. But subsequently it has been shown by Westernbrink and Goudsmith (1938) that the vitamin should be adsorbed from a solution by frankonite, clarit acid clay or

some suitable material in order to concentrate the vitamin and free it from the other impurities. Later on it has been reported by Jowett (1940) that the recovery by the above methods was incomplete and inconsistant and he advocated the use of synthetic zeolite claiming that the recovery of the vitamin is always complete and consistant under controlled conditions. More recently 'The association of vitamin chemists of America' (1947) suggested that the vitamin should be adsorbed in a column containing 'Decalso' after the enzymic digestion of the material and subsequent elution of the vitamin from the adsorbent. Again it has been observed by Roth (1938), Melnick and Field (1939) that the free thiamine can be liberated by enzymic digestion which will set free thiamine from combination with the pyrophosphate. The method of Harris and Wang (1941) which is widely followed for the routine determination of thiamine in biological materials consists of the digestion of the material with papsin and takadiastase at pH 4. 5 for 6 hours while that of Pyke (1940) depends upon the separate digestion of the materials with pepsin and takadiastase for 48 hours at 37°C. During the course of our work with different varieties of food products we found that methods generally followed now for routine work taking into consideration its numerous modifications still offered difficulties and hence it was considered desirable to work out a simple and rapid method of estimation of thiamine in biological materials with considerable accuracy.

Experiment

Extraction and digestion of the material : :—Accurately weigh out into a 250 c. c. conical flask, 5 gm. of the material. Add 75 c. c. of the 0. I N hydrochloric acid and heat for half an hour with occational shaking. Cool the extract to 45°-50°. C and add 5 c. c. of the freshly prepared solution of takadiastase. (1 gm. of solid takadiastase in 20 c. c. of 2. 5 M sodium acetate) Incubate at 45°-50° C for 2 hours and cool the digest to room temperature. Make up the volume to 100 c. c., shake the mixture throughly and filter.

Preliminary washing with isobutanol :—Shake 5 c. c. of the clear filtrate with 5 c. c. isobutanol saturated with water in a centrifuge tube. Centrifuge for 10 minutes to hasten the separation of isobutanol layer. Remove the isobutanol from the centrifuge tube.

Conversion of thiamine to thiochrome : :—Transfer an aliquot of the aqueous layer into a separating funnel. Add 1 c. c. of methanol, one drop or more of potassium ferricyanide depending upon the amount of reducing substances present, 1 c. c. of 30 per cent solution of sodium-hydroxide respectively. Allow the sample to stand for one minute after shaking the separating funnel throughly. Add 15 c. c.of isobutanol saturated with water and shake vigorously for 5 minutes to extract the thiochrome produced completely. Allow the mixture to stand for 10 minutes when the isobutanol layer separates. Draw off the aquous layer. Add 4 c. c. distilled water and shake thoroughly. Wait for 10 minutes when the isobutanol layer separates. Draw off the water and added 2 c. c. of absolute alcohol. Prepare a blank side by side for each experiment, in the same way as described above but do not add potassium ferricyanide.

Measurement of fluorescence : :—Adjust the 'Lumitron' florometer with working quinine sulphate standard solution so that the instrument gives cent per cent transmission using primary and secondary B_1, filters. The percentage adsorption of the sample is then found out and the amount of thiamine present is then calculated from the standard graph prepared with the pure solution of thiamine hydrochloride.

Recovery of the added thiamine in the pulses : ; Thiamine in different amounts was added to different samples of pulses whose thiamine content has been determined side by side in order to learn how far this procedure could recover the added thiamine from the samples. 98 to 100 percent of the added thiamine could be recovered from the samples by this method. The results are presented in table I.

TABLE I

Recovery of the Thiamine added to the samples.

Name	Thiamine added 'γ'	Thiamine recovered 'γ'	Precentage recovered.
Cajunus indicus			
(1)	0	0.30	
(1a)	1.0	1.29	98.4
(1b)	1.0	1.30	100.0
Phaseolus mungo			
1.	0	0.28	
(1a)	2.0	0.25	98.7
(1b)	2.0	0.26	99.0
Phaseolus radiatus			
(1)	0	0.25	
(1a)	3.0	3.21	98.7
(1b)	3.0	3.19	98 1

Thiamine content of different biological materials : :—Thiamine content of differnt biological materials like pulses, cereals, skimed milk, Brewers yeast-tablet, spinach and a scorbutic diet containing gram, barley, caseine, sodium chloride and calcium carbonate, has been determined by this method. The results are given in Table II.

TABLE II.

Thiamine Content of Some Biological Materials.
(Thiamine content in mg. per 100 gm.)

Name	Thiamine	Name	Thiamine
Phaseolus mungo	0.28	Corn	0.22
Phaseolus radiatus	0.26	Brewers' yeast table	0.25
Phaseolus roxburgii	0.25	Skimed milk (powder)	0.21
Cajunus indicus	0.32	Scorbutic diet	0.46
Lens esculenta	0.20	Spinach Sp	0.17
Hordenum Vulgare	0.30	Spinach—Sp	0.10
Cicer Arietinum	0.42	Pisum sativum	0.42

Discussion

Unlike Harris and Wang (1941) and Pyke (1940) the time of enzymic digestion was minimised. The adjustment of pH at different steps were also avoided. The pH of resulting acid extract is such that thiamine is very stable, even when heated in a steam bath for one hour or longer. The enzyme solution which is prepared with 2. 5 M sodium acetate brings the pH of the digest to 4. 5 to 5 so that neither the activity of the enzyme nor the loss of thiamine occured during the process of digestion. Preliminary washing with isobutanol was undertaken in order to remove any foreign fluorescent substances which might interfere with the estimation of thiamine. The adsorption of the vitamin on activated surfaces and its subsequent elution was avoided due to the disadvantages of the absorption techniques as they are tedious and time consuming and may result in serious losses because of the incomplete adsorption or elution of the vitamin. This method of estimation of thiamine seems to be the simplest and easiest of all the existing chemical methods of estimation of thiamine in biological materials.

Summary

1. A rapid chemical method of estimation of thiamine in biological material has been described.

2. The ground material was heated with O. I N hydrochloric acid for half an hour and digested with takadiastase at 45°-50° C for 2 hours. The filtrate thus obtained was shaken with isobutanol and centrifuged. An aliquot of the aqueous centrifugate was converted to thiochrome which was subsequently measured fluorometrically.

3. Thiamine content of some biological substances have been presented.

4. The method seems to be the easiest and simplest of all the chemical methods of estimation of thiamine.

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The Indian Journal of
Physiology ahd Allied Sciences
Vol. III. No. 3, 1949.

HYPOPROTHROMBINAEMIA AND PROTHROMBIN-FORMING FUNCTION OF LIVER OF RATS AFTER ALLOXAN ADMINISTRATION.

By
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Bailey et al (1944), and Lazarow and Palay (1946) have shown that intravenous injection of alloxan produces necrotic foci in the liver. The importance of the liver in controlling the bloodsugar of alloxan-treated animals has been emphasized by Houssay et al (1945). Sen & Sen Gupta (1949) have shown that coagulation time of blood increased after intravenous injection of alloxan. All these suggest that alloxan intereferes with the normal functioning of the liver. It was, therefore, of interest to see the blood prothrombin changes and the prothrombin-forming function of liver after alloxan administration.

Experimental.

Rats were used as it was known that any apparent hypoprothrombinaemia that might have been present before the experiment could not have been due to Vitamin K deficiency, since it was shown by Orla-Jensen et al (1941) that fæces of rats on Vitamin K-free ration, contained Vitamin K.

Three groups of male albino rats weighing between 75 and 120 gm. were taken.

Groups A and B contained 5 rats each and were given 40mg/kg of a 2% solution of alloxan by the tail vein.

Group C contained 2 rats and served as the control.

Prothrombin time were estimated by the modified method of Quick (1938) with diluted (12.5%) plasma (Unger et al 1948).

Prothrombin times of Group A rats were determined 2, 4, 8, 24, 48 and 72 hrs. after alloxan treatment.

Vitamin K "load" test for liver function was carried out with Group B before, during and after intravenous administration of 1.2 mg/kg. Vitamin K daily for two days (Unger et al 1948) Synkavit 'Roche' was used. Liver function test was also carried out with Group C.

Commercial thromboplastin was used in the prothrombin time determinations.

Results.

It was found that alloxan in the dosage given gradually

TABLE. I

GROUP A

Effect of injection of a single diabetogenic dose of alloxan on the diluted (12.5%) plasma prothrombin time of the rat.

No. of Rat	Weight of Rat : gm.	Diluted 12.5% plasma prothrombin time : Seconds						
		Before Alloxan injection	Hours after alloxan injection					
			2	4	8	24	48	72
A 1	75	42	44	44	45	49	54	58
A 2	82	44	45	46	47	52	55	58
A 3	90	42	43	43	45	49	54	57
A 4	112	45	46	47	49	53	57	died
A 5	120	44	45	45	46	49	53	57

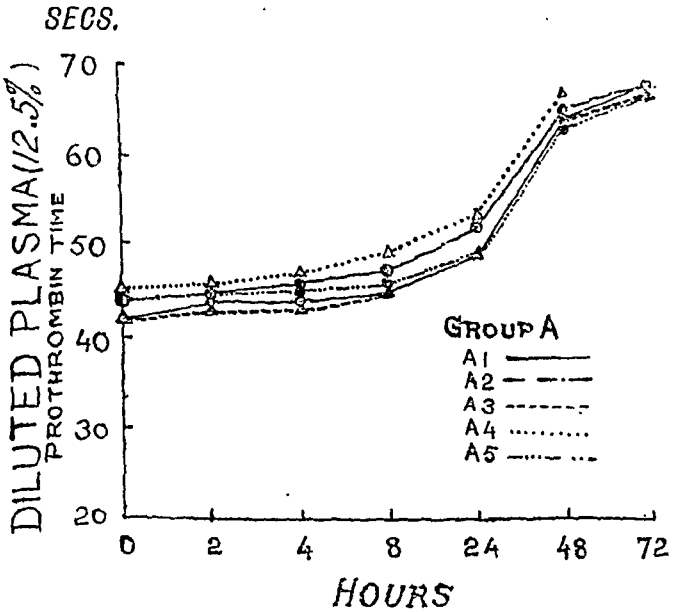

FIG. 1

increased the prothrombin time In some there appeared light haemorrhagic tendency in the eyes after 48 hours. One of these died on the last day of the experimental period. Post mortem examination showed some haemorrhage in the gastrointestinal tract.

Figs. 2 and 3 give the results of liver function test in

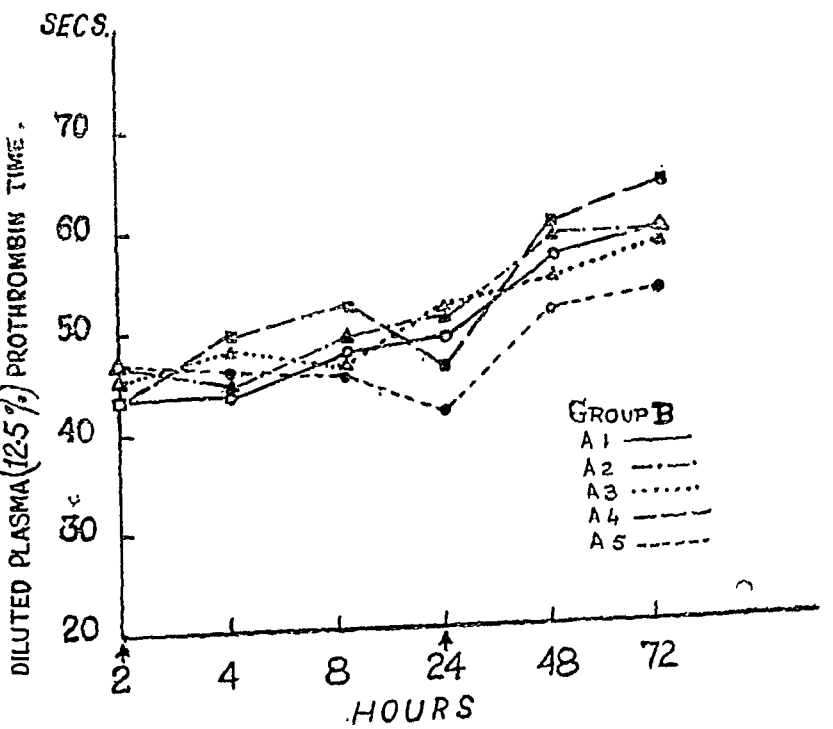

FIG. 2

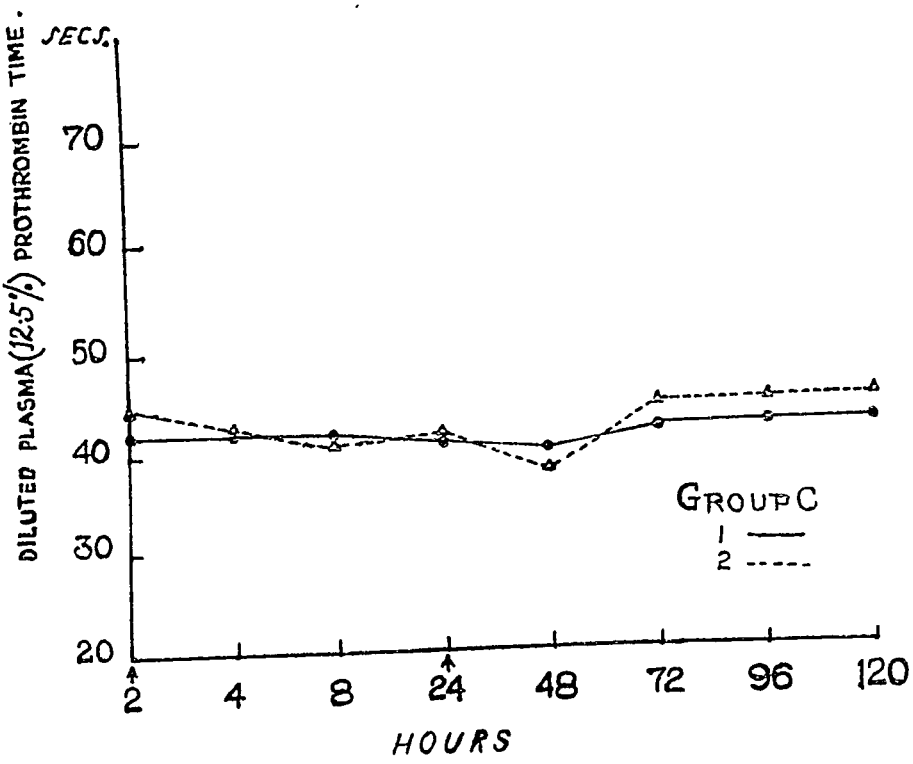

FIG. 3

Groups B and C. It will be seen that while in the control group (group C) practically no change in the prothrombin time was evident there was in every case a prolongation of the prothrombin time in Group B. after Vitamin K administration.

Discussion.

From the table it is evident that blood prothrombin level is diminished after alloxan administration. The capacity of the liver to form prothrombin is definitely lowered (fig. 2.). All the liver function tests are dependent upon some particular function of the liver. It is only appropriate, therefore, that Vitamin K load test judges only the prothrombin-forming function of the liver. The sensitiveness of the test is immensely increased by the use of diluted plasma. (Unger et al, 1948)

According to Braganca et al (1947), sulphathiazole produced hypoprothrombinaemia is possibly dependent upon the age and the weight of the animal. No such relationship was observed

in our experiments with alloxan in which we employed rats of widely different weights.

It is of interest to note here that blood prothrombin is possibly continuously being destroyed and this is perhaps as quickly being replenished by the liver ; moreover, the rate of production and destruction are probably very rapid, so that hypoprothrombinaemia is apparent within two hours after alloxan administration. Definite histological change in the liver has not been demonstrated within so short a period. It may be possible that alloxan combines with the available-SH groups of prothrombin, thus rendering it temporarily inactive. But if it were true, blood prothrombin level should gradually return to normal. The return is perhaps prevented by liver damage which has by this time set in. Another explanation may be that alloxan probably interferes with the enzymatic system that may be responsible for prothrombin formation.

Summary

1. Hypoprothrombinaemia has been produced in the rat by intravenous administration of alloxan.

2. Prothrombin-forming capacity of the liver was found by the Vitamin K load test. The capacity is lowered after alloxan treatment.

3. Susceptibility to alloxan hypoprothrombinaemia has not been found to be different in different age groups,

4. An explanation for the hypoprothrombinæmia is suggested.

Acknowledgement.

We are grateful to Sri P. B. Sen of the Physiology Dept., Calcutta University for some helpful suggestions.

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The Indian Journal of
physiology and Allied Scienees
Vol. III, No. 3, 1949

INDUSTRIAL PREPARATION OF PEPTONE FROM UNMARKETABLE FISH.

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Industrial preparation of peptone from casein, blood, meat and other sources has a good market in foreign countries and increased demand of such imported products indicates that they are gaining popularity in India. So far no attempt has been made in this country to prepare it from fish.

The present investigation was taken up by the authors with a view to determine whether fish can be tapped as an industrial source for the large scale preparation of peptone, where supply is considerably higher than the local consumption.

In Hyderabad, varieties of fish, such as Rohu (Labeo callbasu) and Catla (Catla catla) do not agree with the organoleptic taste of the local people. The absence of facilities for quick transport and lack of arrangement for fish preservation are contributory factors for bringing down the price of fish to a very low level of 20 lbs. at one rupee.

Two varieties of carp, viz., Rohu (Labeo callbasu) and Catla (Catla catla) were selected for the experiment. Their flesh was

minced and mixed with water. The whole mass was digested on a water bath for 3 hours at 60 C with addition of papain as an hydrolytic enzyme at pH 6.8 (it may be noted the pH of local fish muscle plasma is also 6.8).

The difficulties encountered was the appearance of oil globules as a result of heating, which subsequently was ladled out from the top surface in the process of heating. When solid lumps of fish flesh are no longer apparent, it was regarded that the whole mass had undergone complete digestion. It was then heated to boiling point to prevent spoilage and coagulate any undigested protein and then filtered. Another important aspect of boiling was to arrest further hydrolytic cleavage.

The filtrate was completely dried in a vacuum oven at 60°C. It took about 3 hours to bring one pound of the filtrate to a thick syrup before being about finally dried in vacuo. It was removed from the vacuum drier in a state of thick sticky solid of yellowish-brown colour and the last trace of moisture being ultimately removed from this solid lump by keeping it over a bed of calcium chloride enclosed in a specially made air-tight case. Next the crust was pulverized in a special ball mill and stored in air-tight bottles in a cool place. The peptone thus obtained is a highly hygroscopic substance and was completely soluble in water and not precipitated even at full saturation from its solution by ammonium sulphate. It was also not coagulated by heat.

The authors have in view to push the work on peptone from fish down to amino acids stage when proper facilities and laboratory equipments would be available. In the meanwhile, it is worth mentioning that some work had already been done on the determination of amino acids from a certain common edible fish of Bengal by Roy and Sen (1) and from Halibut fish by Osborn and Heyl (2) Table I and II would indicated amino acid values of some fish.

TABLE I.

The figures indicate values in mg. per 100 g. of desicated or fresh fish.

Bengali Name.	Zoological Name,	Desicated and defated fish.				Fresh Fish.			
		CyN	TyN	TtN	Pr	CyN	TyN	TtN	Pr,
Magur	Clarius magur	134	282	116	98.21	23	48	20	16.95
Kai	Anabus scandens	148	317	97	94.55	22	46	14	14.64
Singi	Saccobranchus (Heteropneustes). fossilis	133	270	122	98.29	19	39	18	14.46
Lata	Ophiocephalus puntatus	86	264	92	96.38	12	38	13	14.47
Bata	Cirrhina reba.	135	334	120	96.16	17	43	15	12.81
Bele	Gobius giuris	149	258	92	97.60	21	36	13	14.03
Bhangar	Mugil tade	118	268	114	98.33	16	38	16	14.03
Tangra	Macroneus vittatus.	116	339	91	96.38	16	47	13	13.91
Puti	Barbus sarana	97	284	97	96.38	15	43	15	15.25

CyN—Cystine nitrogen.
TyN—Tyrosine nitrogen.
TtN—Tryptophane nitrogen.
Pr—Protein

TABLE II.

Products of the Hydrolysis of Muscle Proteins in Per Cent of Ash and Moisture-Free Substance.

	Halibut (Hippoglossus hippoglossus)
1. Tyrosine.	2.39
2. Arginine.	6.34
3. Histidine.	2.55
4. Lysine.	7.45
5. Tryptophane.	+

The result of investigation was most encourging and has yielded a product amounting twelve and half pounds in one hundred pound of fish flesh. It is therefore, a product of industrial possibilities in the Hyderabad State.

The importance of peptone in human medicine is very great and such products, as peptonized, meat, milk, powder and tablets are all sources of peptone. The treatment of protein depletion by administration of protein hydrolysates has been successfully demonstrated in many cases during the last Bengal Famine in 1943 and at Belsen Camp after the liberation of Europe. Therefore, the manufacture of peptone as a protein hydrolysate from fish deserves every attention by the Government as well the industrialists.

The authors feel extremely grateful to Dr. A. Karl of Poona on whose valuable suggestions the problem was taken up and to Dr. M. Rahimullah, Fisheries Officer, Department of Fisheries under whose encouragement and care the work was done.

Summary.

Method of preparation of peptone from fish by papain hydrolysis described.

Nutritional possibilities of these fish peptone preparations were suggested from amount of amino acids in them.

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The Indian Journal of Physiology and Allied Sciences

Vol. III OCTOBER. 1949 No.

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Printed and published by
NIBARAN CHANDRA DAS,
Prabasi Press, Calcutta.

THE INDIAN JOURNAL OF PHYSIOLOGY AND ALLIED SCIENCES

JANUARY, 1948

Published by

THE PHYSIOLOGICAL SOCIETY OF INDIA,

CALCUTTA

Price Rs. 10 per volume.

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The Indian Journal of
Physiology and Allied Sciences
Vol. II, No. 1, 1948.

A STUDY ON THE PHARMACOLOGICAL ACTION OF CORCHORIN—A CRYSTALLINE GLUCOSIDE FROM JUTE SEEDS (*CORCHORUS CAPSULARIS*)

By

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(Chemical Laboratory, Presidency College, Calcutta and Physiological Laboratory, Univesity College of Science and Technology, Calcutta.)

Two species of Corchorus are cultivated in Bengal, viz., *C. capsularis* and *C. olitorius*. Indian works on Materia Medica by Chakradatta described the leaf as a remedy for worms, leprosy and deranged liver. In many houses in Bengal, dry jute leaves are stored and used for medicinal purpose under the name of *nalita*. It is also efficacious in certain cattle diseases. According to Dymock the seeds which have a bitter taste possess medicinal properties and are administered in doses of 80 grains in cases of fever, obstruction of abdominal viscera and dysmenorrhoea.

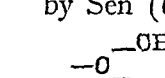
The first pharmacological investigation relating to *Corchorus capsularis* was recorded by Kobert (1), who had received a brown amorphous bitter stuff under the name of corchorin, previously isolated by Tsuno (2), from the firm of Merck for his experiments on animals. He showed that the bitter stuff had strongly toxic properties on frog heart. It appears that Tsuno's (loc. cit) corchorin, which was thought to be an alkaloid, was highly impure substance from which no attempt was made to prepare any essential principle in a pure and crystalline condition. One of the present authors Sen (3) thoroughly studied the chemical composition of *C. capsularis* and isolated an exceedingly bitter glucoside from jute seeds in crystalline form. The glucoside is considered to be the probable source of the reputed physiological action of the seeds which are used to some extent medicinally in India. The bitter principle of jute seeds isolated by Sen (loc. cit) was found to be a

colourless substance crystallising in stout rhombic prisms melting at 174-175°C. It had a molecular formula $C_{22}H_{36}O_8$ and possessed an intensely bitter taste.

The occurrence of two more bitter stuffs, which accompanied corchorin in the alcoholic extract of *C. capsularis* was also noticed by the same author. By fractional crystallisation of the mother liquor residue of corchorin a new crystalline bitter, Corchoritin, having the composition $C_{12}H_{18}O_3$ and melting point 218-220° C was obtained (4). A third crystalline bitter melting at 160° C has recently been isolated from the alcoholic extract of the jute seeds by repeated crystallisation from ethyl acetate and acetone mixture. Their pharmacological action will form the subject matter of next communications.

Saha and Choudhury (5) described a compound under the name of *capsularin* which they had obtained from the leaves of the same plant having the same molecular formula and melting point as the corchorin isolated by Sen (loc. cit) from the seeds of the same plant. But the most interesting thing about corchorin is that it is dextrorotatory $[\alpha]_D = +33.4$ whereas capsularin is leavorotatory $[\alpha]_D = -23.6$. The former is insoluble in dilute mineral acid and is resinified when heated with 2 per cent. sulphuric acid without being hydrolysed whereas the latter dissolves and is easily hydrolysed with 2 per cent. sulphuric acid.

A preliminary study of the chemical structure of the glucoside corchorin by Sen (6) revealed the presence of $\triangle^{\alpha\beta\gamma}$, unsaturated lactone group *viz.*,



the most characteristic pharmacological group of all cardiac poison belonging to digitalis series. It was found to have no hæmolytic action on the red blood corpuscles (7). The present study was planned to explore the effects of administration of corchorin in frog and cat in order to compare its effects with those of digitalis group and further to compare the response of these animals under similar conditions. Experiments were included to ascertain the pharmacological action of the drug and its lethal dose.

Experiments on frog heart.

Frog's heart was perfused with Frog's Ringer at pH 7.4 by the recent technique. The perfusion pressure was kept constant by using Syme's cannula. Kymograph records were obtained by means of heart lever attached to the apex of the ventricle. The observation included the rate, amplitude of contraction and tone of the heart.

The usual procedure of the experiment was to perfuse with normal saline solution until a constant rate, tone and amplitude had been established. The hearts of the frog were perfused alternately with Ringer solution at pH 7.4 and with solution of corchorin in Ringer at different concentration and at the same pH. The perfusion was continued for three to five minutes and several perfusions were made on each heart.

The effect on a considerable number of frogs were studied in order to obtain average results and minimize the natural variability. The number of frogs used with several concentration tested are detailed in the following table.

Concentration of corchorin 1 in ..	Number of frogs	Total number of perfusions.
500,000 in Ringer's solution	10	20
250,000 „ „	7	18
200,000 „ „	6	12
150,000 „ „	7	10
100,000 „ „	7	15
50,000 „ „	8	18
20,000 „ „	6	10
10,000 „ „	9	12
5,000 „ „	6	18
1,000 „ „	5	10

Experiments on Cat.

Blood pressure and respiration of cat anesthetised with chloralose was recorded on the Kymograph in the usual way. When the respiration and blood pressure become normal after connecting the Mary's tambur with the trachæa and the carotid artery with the recording manometer the drug was administered by intravenous injection. For this purpose left femoral vein was dissected out and a venous cannula was introduced into it. The cannula was connected to a burette by means of a narrow rubber tube. The burette contained normal saline. Whenever any drug was required to be introduced the hypodermic syringe containing the desired amount of the drug was injected into the wall of the rubber tube by means of the needle of the syringe, at a slow rate, the time of the application of the drug being recorded. Each time the drug was introduced the results were noted and finally the minimal lethal dose was also found out.

It was found that with different dilutions such as 1 in 50,000, 1 in 20,000, 1 in 10,000, 1 in 1,000 and 1 in 100, there was a graded lowering of blood pressure in each case. The saturated solution (*i.e.*, 0.03 per cent. in normal saline—4 c.c. intravenous) caused a total failure of heart beat and stoppage in systole.

A record of 1 in 10,000 dilution is shown in Figure 5.

Results.

The method of tabulating the results is as follows:—

(1) The experiments of the same nature were put in one group, (2) The chronological perfusions on each heart were grouped, for instance, all of the perfusions with a certain concentration on each heart were grouped This would make the conditions more comparable in case the heart suffered any permanent injury from the perfusion. In fact the results were essentially identical by both methods of tabulation, so that they need not be discussed separately.

Figure (1) shows stimulation of amplitude and rate per minute The tone was also increased (Fig. 2) with all concentrations. But with concentration of 1 in 10,000 the stimulant action on heart was noticed which was stable, the amplitude was increased with the increase in the rate of frequency.

The stimulation was greatest at about the concentration of 1 in 10,000 and there was much improvement progressively with dilution of the glucoside, but corchorin shows some toxic action with saturated solutions in Ringer (Fig. 1). In strongest solution the heart shows a stimulation followed by depression and ultimate stoppage of heart in systole. The drug is very effective in fatigued heart, and the results shows that almost failing heart revived and remained beating with normal tone and frequency for a long period (Fig. 3).

With concentration of 1 in 10,000 the frequency and amplitude of heart and tone are increased. With a dilution of 1 in 1,000 sometimes cardiac response consists of heart block and groupings (Fig. 4) lasting for a short period, followed by progressive partial recovery. Complete recovery does not occur until drug-free Ringer's solution is substituted. The heart block is not due to vagus stimulation, for it is not influenced by the application of atropine either before or during the disturbance.

Statistical analysis of the data.

The average increase in frequencies *i.e.*, number of heart beats per minute and in amplitude of contraction with various doses is shown in Table (A) and logarithim graphs. The effect in each case has been found to be statistically significant. The figures of Table (A) and logarithim graphs exhibit a slight irregular relationship between effect and dose. This is perhaps due to the fact that in the present series of experiments we have not been able to control or eliminate other factors such as seasonal variation and normal frequency or amplitude of the frog heart, which also influence the rate of change of frequencies or amplitude.

The effect of the drug on the tone of the heart was also examined and the proportion of cases in which increase or slight increase in tone was noticed is given in Table (B). With regard to tone also we find that corchorin has a stimulant action with all doses. So to sum up, the following points may be taken into consideration to explain the variations in the stimulant action in different dilutions.

(1) Seasonal variations of the frog heart.

(2) The physiological variability.

(3) Normal frequencies and amplitude of frog heart vary in each experiment, and they may have certain effect in the ultimate results.

TABLE A

Average increase in frequency and amplitude for different doses of corchorin.

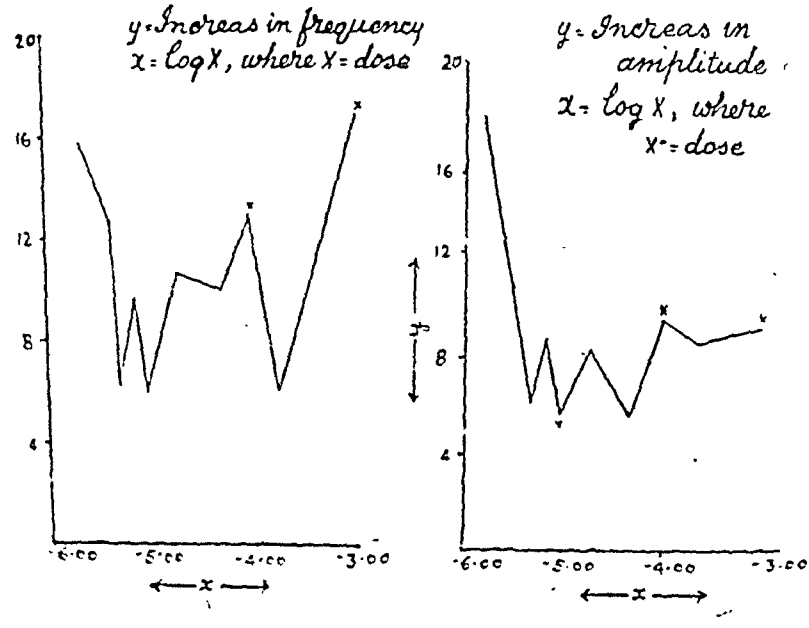
Dose.	First Perfusion.			Second Perfusion.		
	No. of Frogs.	Frequency.	Amplitude.		Frequency.	Amplitude.
1 in 500000	10	15.80	17.10	9	18.89	20.22
1 in 250000	7	12.71	9.14	7	9.43	9.57
1 in 50000	6	6.17	5.50	6	5.17	4.83
1 in 20000	7	9.86	8.00	3	9.67	4.67
1 in 10000	7	6.00	5.14	5	6.60	7.40
1 in 5000	8	10.75	7.62	8	10.12	7.87
1 in 1000	6	10.00	5.17	3	10.67	7.67
1 in 200000	9	12.89	8.89	7	15.86	10.43
1 in 150000	6	6.17	8.00	6	8.17	8.33
1 in 100000	5	17.00	8.60	5	18.00	8.20

TABLE B

Proportion of cases showing increase or slight increase in tone of heart.

Dose.	No. of Frogs.	Proportion.
1 in 500000	10	1.00
1 in 250000	7	1.00
1 in 200000	6	0.50
1 in 150000	7	0.71
1 in 100000	7	0.57
1 in 50000	8	0.75
1 in 20000	6	0.83
1 in 10000	9	0.78
1 in 5000	6	0.84
1 in 1000	5	0.60

LOGARITHMIC GRAPHS



SUMMARY

It is found that very small doses of corchorin, a crystalline glucoside from jute seeds—Corchorus capsularis—in Ringer's solution (1 in 10,000) has a marked stimulating action on heart resembling in some respect the digitalis group of drugs. It has also some action on the blood pressure.

Further work is in progress.

The authors are indebted to Mr. Prasad Kumar Banerjee, M.Sc., of the Statistical Laboratory, Presidency College for the advice concerning the analysis of the data and wish to express their sincere thanks to Prof. S. N. Bose, for the interest he has taken during the progress of the work.

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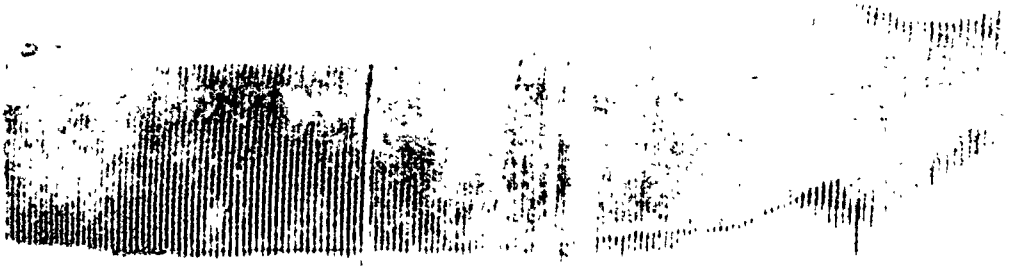

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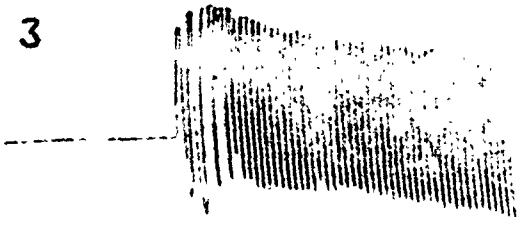

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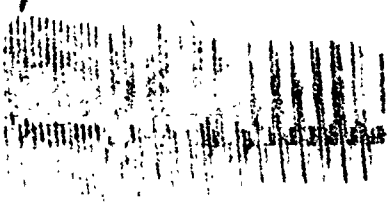

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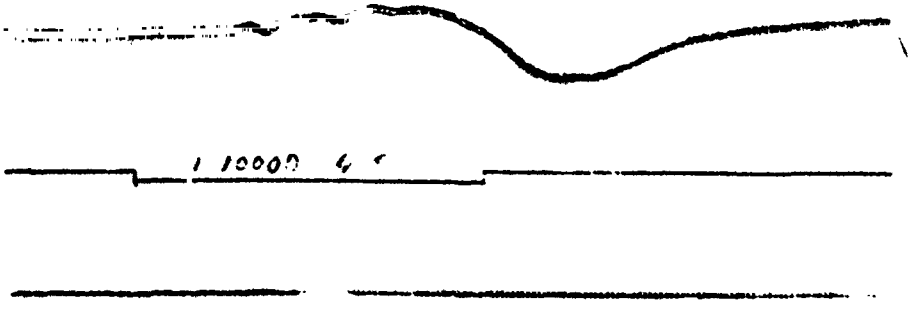

The Indian Journal of
Physiology and Allied Sciences
Vol. II, No. 1, 1948.

BIOLOGICAL ASSAY OF ADRENALINE

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Adrenaline, particularly when it is present in very small quantities is subjected to biological assay to find out whether it has full activity of the Reference Standard Substance.* The assay is usually carried out by finding doses of the standard and unknown which produce equal, submaximal rises in blood pressure in spinal cats[1] or in anæsthetised, atropinised dogs[2]. The disadvantages of this method have been discussed by Thomson[3]. Chance variations in response and changing sensitivity to the drug as the experiment progresses introduce complication in the experiment and difficulty in the interpretation of the data, which then become largely a matter of the judgment and experience of the individual assayist. Although it is possible for an experienced assayist to estimate potency to within 5 or 6 per cent.[1], by repeated comparison of the standard and unknown, the number of doses that would be necessary to obtain this accuracy in any instance is indeterminate since it depends on the sensitivity and variability of the animal being used and on the judgment and experience of the assayist. Further, the method fails to provide for the utilization of all the data obtained and is therefore considered inefficient[4].

To obviate these defects and difficulties, modifications of the current methods have been suggested, utilising, in the main, modern experimental design and method of interpretation. The method suggested by Thomson[4] is stated to provide a degree of accuracy seldom attained by means of a biological procedure. However, it is considered by Knudsen *et al*[4] to be too laborious and time-consuming to be practical for routine work. These workers have presented a method based on the use of a balanced experimental design and the use of a simple chart and nomograph for making the necessary calculations, which is claimed to be simple and time-sparing and yet possessing a degree of accuracy which should make it generally useful. The present communication contains our observations on adrenaline assay carried out with the method developed by Knudsen *et al*[4]. These workers used anæsthetised, atropinised dogs as directed in U.S.P[2]. In our experiments, spinal cats, as is the practice in this laboratory, were used instead.

* There is no 'International Standard' for Adrenaline. Adrenaline B. P. (satisfying all tests for chemical purity) is used as a Reference Standard.

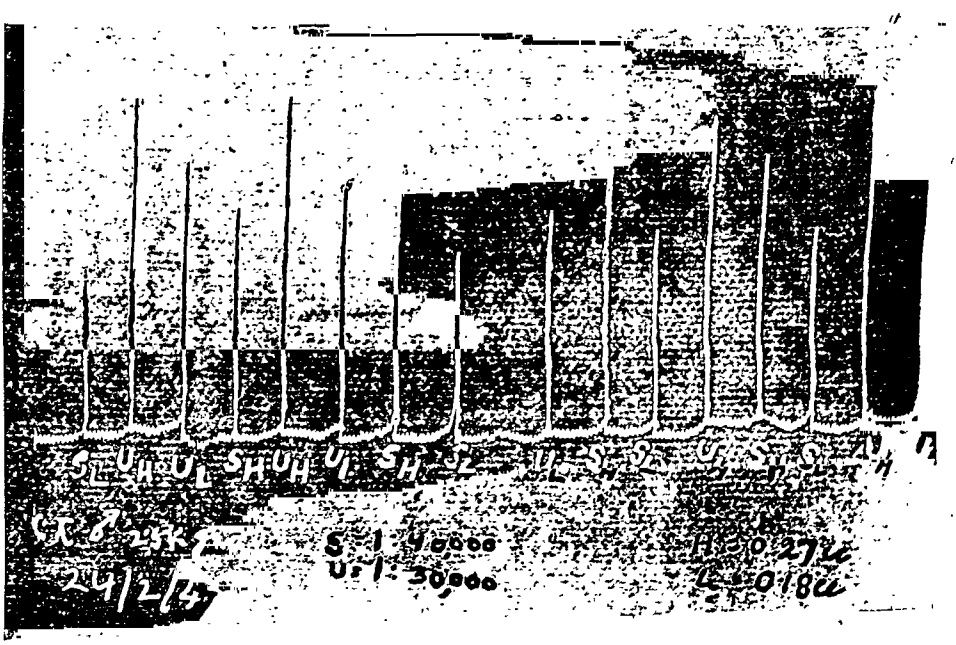
Experimental:

Full-grown male cats weighing between 2 to 4 kg. were anæsthesied with ether and the spinal cord divided and prepared for the record of carotid blood pressure[1].

A dose of the Reference Standard Adrenaline solution usually diluted to 1:40,000 to 1:60,000 which gives a blood pressure rise of approximately 40 mm. Hg. was selected. This dose represented the low dose of the standard (SL). Sample solutions of different concentrations made from the Reference Standard Adrenaline were prepared and tested. These represented the unknown solutions and these were so diluted as to be theoretically equal in potency to the diluted solutions. A volume of the diluted unknown solution equal to SL represented the low dose of the unknown, UL, and 1.5 times this volume the high dose, UH and SH. Each of these four doses was administered four times in order determined by assigning the various doses at random to a 4×4 latin square, an example of which is shown in Graph I. The injections were made into the saphenous vein at five minute intervals. Each of the sixteen responses as indicated by the rise of blood pressure was measured to the nearest millimetre.

GRAPH I

Record of blood pressure in an individual assay.



Nine solutions of known potency prepared from the Reference Standard Adrenaline was tested and the data treated by the methods developed by Knudsen et al[1]. The true potencies of the solutions under test were not disclosed to the assayist until the results of the assays had been calculated. The results of the assays are given in Table I.

TABLE I.

Assays of solutions of known potency.

Assay No.	True Potency. per cent	Estimated Potency. per cent	Standard Error. per cent	Actual Error. per cent
1	71.4	78.0	4.83	9.24
2	66.6	71.5	5.93	7.35
3	57.1	61.1	5.13	7.00
4	80.0	72.5	9.05	9.37
5	114.2	113.0	11.86	1.05
6	142.8	140.0	7.00	1.96
7	100.0	99.0	2.18	1.00
8	88.8	93.0	2.28	4.72
9	133.3	143.0	10.72	7.27
Mean			7.7	5.44

DISCUSSION

In our series of nine assays the results obtained (average actual error 5.4 per cent.) compare favourably with those of Knudsen *et al*[1], who reported an average actual error of 5.0 per cent. in an identical series of assays. This shows the easy reproducibility of the result in different laboratories. However, it appears that the use of spinal cats, as in our experiments, instead of anæsthetised, atropinised dogs used by Knudsen *et al*[1] does not produce any significant difference in the results obtained. The method is simple and more or less mechanical requiring little judgment on the part of the assayist as the doses of the standard and the sample are fixed and repeated every five minute in a definite sequence throughout the experiment, irrespective of the degree of response produced. It has been our experience working in the spinal cats that the blood pressure does not always come to the base line after 5 minutes following an injection of adrenaline. However, we have repeated the injections every five minute according to the directions contained in the paper. The paper does not also contain any direction in regard to another important point, *viz.*, what course is to be adopted in case of interruption of the experiment, as for instance, by the formation of a clot—whether the assay should be started afresh from the first injection of a group or from the point where the experiment was interrupted. This should be important in view of the fixed interval between the injections and the fixed doses to be employed. Fortunately, such an exigency did not arise in course of our experiment and we are thus unable to comment on this.

The degree of accuracy obtained by routine method[1] followed in this laboratory does not differ significantly from that obtained with the method under investigation. The method is time-consuming, as even using a five minute interval about 1½ hour is required for completion of one assay. Further it is not possible (taking into account the number of doses to be administered, 16 for each assay) to assay more than 3 samples in one animal; on the other hand the routine method followed in the laboratory permits the assay of at least double this number of samples.

Taking into consideration all these facts, the method advocated by Knudsen et al[1] is not a suitable one for routine work, especially in a laboratory from which a large number of samples are to be reported upon. The

however be employed with advantage in doubtful cases for checking the result as it largely avoids the personal factor of the assayist.

Summary and Conclusions.

(*i*) Assay of adrenaline has been carried out by the method developed by Knudsen *et al*[4], calculating the results from the chart and nomograph supplied.

(*ii*) Spinal cats were used instead of anæsthetised, atropinised dogs.

(*iii*) Results of nine assays (average actual error 5.4 per cent.) reported in this paper compare favourably with those of Knudsen et al[4], in an identical number of assays (average actual error 5.0 per cent.). This shows the easy reproducibility of the results in different laboratories.

(*iv*) The method is time-consuming when contrasted to Burn's method which is followed in this laboratory and thus cannot be accepted as a routine procedure in a laboratory from where a large number of samples are to be reported. The level of accuracy is not such as to outweigh the consideration of time factor.

(*v*) The supreme merit of the method is that it is simple and more or less mechanical and largely avoids the personal factor of the assayist and as such it can be utilised with advantage in doubtful cases to check up the result.

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The Indian Journal of
Physiology and Allied Sciences
Vol. II, No. 1, 1948.

INFULENCE OF TEMPERATURE VARIATIONS ON THE ACTIVITIES OF THE SMALL INTESTINE AND ON THE ACTION OF CERTAIN DRUGS ON IT.

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The response of the mammalian tissue is best at the optimum physiological conditions, one very important item of which is the temperature. All the warm-blooded animals have not the same temperature; thus the human skin temperature is 37°C, the temperature of the rabit is 38°C while that of the rat is 40°C. The same temperature cannot therefore be ideal for experiments on the tissues of different animals. This is a very important point and must be borne in mind while carrying on experiments on mammalian tissues. Again, there is individual variation in the animal of the same species. Hence in order to obtain the best physiological response from an excised tissue or organ it is essential that the temperature of the experiments must be maintained at least at the same level as that of a normal animal of that species. This is, however, more or less known. It is now necessary that some information should be available about the temperature at which a drug can act best. In certain quarters there is a hearsay against the administration of quinine during the high pyrexial stage in Malarial fever on grounds of its deleterious effect but as there is no experimental proof in its favour it has not attracted much notice. After all, very little work seems to have been done to show if there is any difference in the activity of a drug at different temperature. Temperature is known to bring changes in the outward manifestations *e.g.*, the rate of pulse, respiration, vascularity of a part etc. but studies on the efficacy of a drug at different temperature is significantly small. This is very important from the clinical standpoint. Fuhrman (1946) recorded that the fatal dose of colchicine for a frog at 20°C was 1.3 mg. per gm. body weight, whereas at 30°C it was only 0.003 mg. Acetylcholine proved in his hand more toxic to mouse than to a frog when the latter maintained a temperature of 17°C less than that of the former. Under similar condition adrenaline proved sixty times more toxic to the frog. Langlois (1897) recorded greater persistence of the rapid rate of the turtle heart by epinephrine at 15°C than at 37°C. The response of the nictitating membrane to adrenaline increased progressively as the temperature was reduced (Fuhrman et al-1944). Blaschko & Schlossman (1938) obtained a more marked response to adrenaline in the rabbit's gut at 30°C. It is thus found that intensity of the response to drugs not only depends on the temperature but also on the peculiarity of the tissue of animals and nature of the drug. It has therefore been intended to take up the investigation to show if temperature produces a change in the intensity of action of drugs

on the small intestine. In the present case two drugs, acetylcholine and adrenaline could be tried at different temperature in addition to the effect of temperature itself on the gut. It seems that the subject has not gained sufficient attention probably because man is a homoiothermal animal and as such he cannot show wide variation in temperature. But this is not wholly true. Talbott (1941) and Reincke as early as 1875 showed that man can stand a range of variation of 20° in the temperature and he can withstand the lowest temperature of 23°C.

Experimental Methods

A simple plain muscle bath and Tyrode's solution (composition NaCl-0.80 gm., KCl-0.02 gm., CaCl2-0.02 gm., NaHCO3-0.10 gm., glucose-0.10 gm., MgCl2-0.010 gm., NaH2PO4-0.005 gm., and water to 100 ml.) have been used. Rabbit was killed by bleeding after the animal was stunned with a blow on the medulla. Abdomen of the animal was opened and the gut removed with the help of a pair of forceps and scissors without touching with the fingers or pulling on the gut itself. A suitable piece of ileum about 2 cm. in length was suspended in the bath vertically and a thread stretched from its upper end to the lever to record the movement of the longitudinal coat. Movement of the circular muscle was recorded by air displacement method (modified Trendelenburg-1917) with the help of McDowall's recorder. The movement were so registered that the upstroke and the downstroke represented contraction and relaxation respectively. Oxygen was passed through a specially made conduit pipe so that the bubbles might not appreciably disturb the gut. Drugs were added in solution made up in distilled water with the help of a pipette not directly on the piece of gut but to the bath solution. Temperature of the bath was changed by adding hot or cold water even ice to the outer jacket of the bath. An interval of 5 minutes was always allowed to enable the bath to acquire the requisite temperature before any record was taken. A thermometer inside the bath recorded the temperature.

Results

A simple lowering of temperature from 42° to 32°C produced several changes in the movement of the gut. Temperature was reduced by two degrees at a time and the changes that occurred were recorded (Figs. 1 & 2). It will be apparent from the tracing that rate, amplitude, rhythm and the tone varied with the change in temperature. These variations are as follows, (1) rate became slower as the temperature was lowered, (2) amplitude of movement became smaller with a decrease in the temperature. Slower rate and decreased amplitude were almost constant changes as the temperature was reduced. (3) Rhythm did not show a constant change like the former two. Usually it became irregular below 36°C, and (4) Generally the tone showed a rise as the temperature came down. Tone was measured from the spontaneous alteration of the base line. Elevation and depression in the base line indicated a rise and fall of tone respectively.

ACETYLCHOLINE AND ADRENALINE

The former stimulates, the latter inhibits. In these experiments at a higher temperature (42°C) the gut showed stimulation to acetylcholine (1.0 γ g.) which was characterised only by an increase in the amplitude of contraction, but as the temperature was lowered the stimulation was by an increase in tone with or without increase in the amplitude. At 32°C it gave the highest response. (Fig. 1).

In case of adrenaline the same dose (1.$^{\alpha}$ g.) produced in the gut a gradually more marked loss of tone and inhibition in the movement at a

lower temperature, the maximum effect being obtained at 32°C (Fig. 2). At the lower temperature the gut required also more time to pick up its original rhythm. The tracings will illustrate the changes due to acetylcholine and adrenaline on the top of the changes brought on by simple lowering of the temperature.

Discussion

The changes in the rate, rhythm etc. of the contraction of the small intestine owing to a difference of temperature is well known. This is to a certain extent analogous to the changes in the actions of the heart when an individual suffers from a rise of body temperature. Although the changes are well known the cause for such changes is far from clear. The small intestine has been found highly sensitive to mechanical and physical stimuli and it appears that the gut is similarly sensitive to thermal stimuli. It may also be due to an increase in the metabolic rate which is always stimulated by a rise in the temperature. But whatever may be the real cause the changes appear to be quite physiological. The enhanced rate and amplitude etc. of the contraction of the gut at high temperature is an unequivocal attempt to expel its contents readily which is attended with loss of body heat. This is probably the reason of the involuntary passage of urine and fæces in the clinical entity "Heat exhaustion."

These are the probable reason for the changes in the normal activities of the gut but the response by the gut to the drugs acetylcholine and adrenaline at a temperature much below normal is obscure. The results suggest that either the two drugs are more toxic to the intestinal tissue at a lower temperature or the drugs gradually undergo oxidation or some other changes into less active substance when the temperature is raised. In fact, more work is needed to settle this question; at the same time it emphasises the necessity of working with drugs to determine the temperature at which they exert their maximum effect. Results of such studies promise considerably valuable application in the clinical medicine.

I record my thanks to Professor R. J. S. McDowall, M.D., D.Sc., F.R.C.P. of the King's College, London, for the facility he gave me in conducting this study.

SUMMARY

1. Variation in the rate, rhythm, amplitude and tone of the rabbit's gut owing to a change in the temperature confirms the previous findings.

2. In the rabbit response of the gut to acetylcholine and adrenaline seems to be best at 32°C and not at the normal temperature (38°C).

3. Experiments show that the action of drugs (acetylcholine and adrenaline) on the intestine is not equally intense at all temperature, and suggest that similar variation in the intensity of action according to the temperature is probable with other drugs as well and on the different tissues. The fact illustrates the necessity of more work on the line.

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Fig. 1. Rabbit's ileum. Increased rate and amplitude at 42°C while irregular rhythm and increased tone at lower temperature (32°C). Response to acetylcholine (1.0 ug.) at Ac at 32°C. Upper and the lower tracings indicate the movement of the circular and the longitudinal coat respectively.

Fig. 2. Rabbit's ileum. At 42°C the gut showed less tone and increased rate At 32°C there was less amplitude and more irregularity. It also shows the maximum loss of movement and inhibition at 32°C by adrenaline (1.0 mg.) at A.

The Indian Journal of
Physiology and Allied Sciences
Vol. II, No. 1, 1948.

VITAMIN C CONTENT OF SOME ORDINARY FOODS BEFORE AND AFTER COOKING

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In the course of determination of the optimum requirements of Vitamin C (Basu & Roy—1941) it was found necessary to determine the available Vitamin C in foods after cooking. The present investigation was, therefore, undertaken and was limited to such foods as used to be taken by the subjects under investigation for the determination of their optimum requirements of Vitamin C.

EXPERIMENTAL

A weighed amount of the sample of food, with or without peel as mentioned in the chart given below, is macerated thoroughly in a glass basin with a mixture of HCl (4%) and meta-phosphoric acid (2%) and sand (previously washed with dil HCl & dried). The whole of this macerated substance is then transferred with the help of the acid mixture to a flask, fitted up with a reflux condenser, and then boiled for 10 mins. in a current of H_2S. Boiling releases ascorbic acid from its combination and H_2S prevents oxidation of free and released ascorbic acid and reduces de-hydro-ascorbic acid to ascorbic acid. The passage of H_2S is continued for 5 mins., after boiling is stopped. H_2S is then expelled by the passage of a strong current of CO_2 for about ½ an hour. The whole mixture is then made up to a definite volume by the acid mixture. Two equal aliquot portions of this fluid extract are then titrated with Tillman's dye but one of them was first treated with Hex-oxidase, prepared from drumstick juice (Sreenivasam—1937). The difference between the two titre values gives the amount of total ascorbic acid in the aliquot portion and then, by calculation, the total ascorbic acid in the weighed sample or in 100 g of the substance is obtained.

The free ascorbic acid content is obtained in the same way without boiling the macerated mixture in a flask.

The difference between the total ascorbic acid and the free ascorbic acid gives the amount of dehydro-ascorbic acid and combined ascorbic acid.

After cooking which is expected to release all the ascorbic acid from its combination, only free acid was estimated. The de-hydro acid, if any, would occur in minute amounts and a large part of it would be destroyed by cooking. The periods of frying and boiling varied according to the requirements.

RESULTS

The results of estimation are given in the appended table.

DISCUSSION

Of the 21 substances analysed, six had their vitamin C content reduced by cooking to the extent of nearly 80%, one by 68%, two by 52%, eight by 30 to 40%, three by 20 to 24% and one by 10% only. This difference in the extent of destruction of vitamin C by cooking is not influenced in any way by the release of ascorbic acid from its combination during cooking,

for pumpkin which contains only free ascorbic acid undergoes destruction of its vitamin C to the extent of 83%, whereas plaintain flower which contains the maximum amount of de-hydro and combined ascorbic acid, has its vitamin C destroyed to the extent of 82%. It is apparently the presence of protective substances in these foods, which causes the difference in destruction of their vitamin C during cooking. The amount, the nature and the mode of action of these protective substances require to be thoroughly elucidated.

CONCLUSION

This investigation shows clearly that even after prolonged heat treatment of various foods to which they are subjected during cooking, sufficient vitamin C is still left in some of them and is not completely destroyed in any of them. This fact accounts for the absence of pronounced symptoms of vitamin C deficiency in persons who do not usually take and raw foods.

THE DIGESTIBILITY OF SOYABEAN ASSOCIATED WITH CARICA PAPYA (ALBINO RAT FEEDING EXPERIMENTS)

By

PROF. N. C. NAG.

Of all the legumes a great food value was ascribed to Soybean, and for last few years various workers in the field published hundreds of papers dealing with the food value of soyabean. The soyabean is cultivated through out the world now, previously China was the only place where it was cultivated. The high protein content led scientist to study its food value. It was found that the bad odour and indigestibility was one of the cause of rejecting it as common food. After great war in 1920. Soyabean dishes were served in hotels and restaurants, in the United Kingdom.

The cultivation and yield was studied by the present author. The composition was analysed of different specimens collected from various sources. The Falta grown variety and Sodepur grown seeds have the same composition.

The presence of high percentage of edible oil and proteins combined with low percentage of carbohydrate in Soyabean, militates against its value as completely and easily assimilable food. Rats (albinos) reared on Soyabean alone have shorter, span of life and somewhat lesser weight than those reared on chola (*Cicer aretinum*) (which by the way with only 4.2 per cent oil and 19 per cent protein and 63 per cent carbohydrate) proved to be a complete food in itself. Chola contains a large quantity of vitamin in comparison to soyabean. A mixed diet, however when balanced with carbohydrate and other sources of vitamins might avoid this risk.

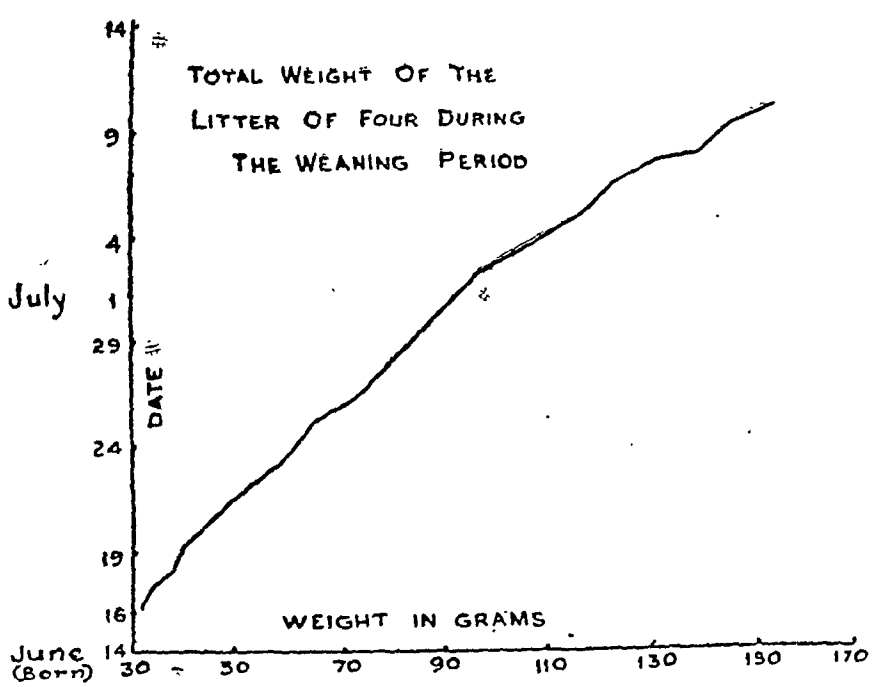
While working with Ereptase and Peptase of Carica Papaya on different types of vegetable protein I found that Soyabean protein is acted

upon by those enzymes and broken up into simpler cleavage products. So I started the work with Carica Papaya and Soyabean and collected data regarding growth and development and fertility of albino rats.

Rats generally develop and grow fatter during weaning period in normal diet. But when Soya bean diet was adminstered during that period the development was less and there was lowering of weight, instead of increase. From the Graph II it will be noted that a group of Female rats on chola diet gained much in weight than on a similar group of rats on Soyabean diet. Finding difficulties to co-relate the cause of want of nutrition in Soyabean diet, different methods were devised to increase the assimilation of Soyabean by frying and powdering it in a mortar. This also was not successful because as the animals showed aversion to this quality of diet. Boiled Soyabean was also given to young rats, but no better results were recorded.

Finally when Soya bean meal was boiled with a small quantity of green carica paypa (say 1 gram of Papya for 25 gram of Soyabean), the Soya-bean became more digestible. The graph II showing that after administration of Soyabean boiled with papaya on 21st July, the rise in weight was steady (Ms & Ms) whereas when fried beans were given progress was irregular. Besides showing recovery and lesser growth, they became fertile a little earlier than usual. They laid their first litters on the 86 and 87th and 92nd days of their age. In chola and other diets rats laid their first litter on the 110th day.

GRAPH I

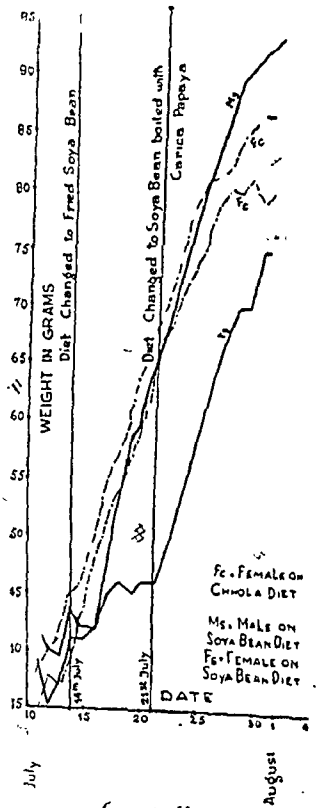


A series of young rats of average weight and age was selected and fed on boiled Soyabean-Papaya preparations. The effect was good and uniform From the graph it will be noticed that in case of male and female rats the improvement in growth was regular .

In a particular case of an ill fed pair of albino-rat with chola diet which gave birth to a litter containing nine which died in a day or two showing under-development of joints of the lower limbs, and spinal columm. When animals became pregnant 2nd time, the young ones which came out the same phenomena were observed and all the young ones died within a few days. The animals were then fed on boiled Soyabean Papaya diet, the third pregency gave birth to a litter of young rats all of which survived.

It can be mentioned here that the balanced diet along with soyabean papaya gave a very good result regarding the growth and development of albino rats.

I am indebted to Dr. Sudhir Sanyal of Calcutta Bacteriological Institute for the supply of Albino rats. Thanks to my grand-nephew Sriman Arup Bose student of University college of Science for plotting up the graphs.



Graph II

CONSTITUENTS OF KANKROL

(Momordica cochinchinensis Spreng Momordica dioica-Roxb. and Kankrol of unknown variety.)

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There are three varieties of Kankrols available in the Calcutta markets. They are analysed for determination of their proximate principles. In the absence of botanical name for one of them, a brief description of the fruits of these three varieties are given, so that they may be correctly Indentified.

Variety 1. *Momordica Cochinchinensis-Spreng.*

The plant is either cultivated or it grows wild in hilly tracts of Bengal, Burma and Deccan Peninsula. The fruits which are slightly bitter are consumed as vegetables. The ovoid fruits are four to five inches in length and about three inches in breadth and are covered with soft spikes, all over the surface. The seeds of the fruit are polyhedral, flattened and corrogated on the margin.

Variety 2. *Momordica dioica. Roxb.*

The plant is cultivated all over India. The ellipsoid fruits are one to three inches in length and about one inch in breadth. They are also densely covered with soft and slender spikes, but are decidedly less bitter than M. cochinchinensis. The seeds though of similar shape to that of M. cochinchinensis are considerably smaller.

Variety 3. *Botanical name—Unknown.*

The plants are cultivated all over northern India. The fruits look almost like M. *dioica,* but are smaller and more round in shape. The seeds unlike those of the Momordica of varieties one and two, are round smooth like peas. The fruits resembel Momordica dioica (Roxb) in taste. The botanical name of this variety could not be ascertained and the matter was referred to the Nutrition Board of the Govenrment of Bengal. They after consulting various scientific institutions of Calcutta and Lyallpur came to the conclusion that probably a new name has got to be coined. As this is not the scope of this paper the question is left in the hand of proper scientific institutions.

The confusion caused by such unsettled nomenclature is considerable. The Behar variety, for instance, is mentioned by Mitra, Mitra (1) to be Momordica cochinchinensis but according to Hain (2) only M. dioica is available in the province. The Kangra valley variety has been indentified by Lander (3) as Momordica Muricata, but according to the findings of the Nutrition Committee (4), it is probably the unidentified variety, because Momordica Muricata is not Kankarol or Kankora but a smaller variety of Karela. These facts show a necessity of reopening the entire question.

A synopsis of the report of analysis by a group of workers is given below along with the results of varieties of Kankrol analysed by the author.

Lander (3)—*Momordica Muricata* (?)

Moisture	.. 84.2	Insoluble residue	0.084	Calorific value is	52.1
Protein	.. 2.28	Ab2 O3 ..	0.007	cal./100g.	
Fat.	.. 1.89	Calcium. ..	0.021	Carotene ..	20.6 mg.
Carbohydrates	.. 6.21	MgO. ..	0.004	Vit. C. ..	224.4 mg.
		MnO.	trace		
Fibre	.. 4.07	P2 O3 ..	0.108		
Ash.	.. 1.35	K2 O. ..	0.500		

Mitra & Mitra (1) *Momordica chochinchinensis.*

Moisture	.. 90.43	Ash. ..	0.9	Crude fibre ..	1.57
Protein.	.. 0.58	Carbohyd-rates ..	6.44	Ca O. ..	0.027
Fat.	.. 0.08			P2 O3 ..	0.38

Paul and Chakervarty—Momordica dioica (5).

Fe.	.. 0.0011%	Protein	.. 1.02%
Ca. O.	.. 0.0430		
P2 O3.	.. 0.4270		

Mitra, Mitra, and Roy (7).

Momordica cochinchinensis.	Carotene. ..	0.4 mg.	Ascorbic acid. ..	65.0
Small country variety.	Carotene ..	1.8 „	Vitamin C. ..	146.9

Mitra (6). *Momordica Cochinchinensis.*

Moisture.	.. 84.09	Carbohyderates ..	5.69	Calcium. ..	0.021
Protein.	.. 2.61	Fibre. ..	5.93	P2 O3 ..	0.148
Fat.	.. 0.66	Ash ..	1.02	Iron. ..	0.59
Calories.	.. 40.2 per 100 grams.				

As every part of the fruit is considered edible in these three varieties, an analysis of the whole fruit was conducted. Representative samples of the fruits in all stages of development were collected for analysis. The following is the data of the analysis.

	Wild Variety Momordica Cochinchinensis.	Medium Momordica Dioica.	Small Un-named variety.
Moisture	.. 91.8 %	87.7 %	38.1 %
Protein	.. 1.392	1.32	1.26
Ash.	.. 0.687	0.670	0.710
Lipoids	.. 0.446	0.365	0.625
Non-reducing sugar.	.. 0.370	0.460	0.400
Reducing Sugar.	.. 1.800	2.230	1.500
Starch.	.. 1.300	2.010	1.700
Fibre.	.. 2.205	5.225	5.705

Comparing the analytical data of the present work, no marked differences can be observed between these three varieties. Only the lipoid content is slightly high in the small variety and carbohyderate content in Dioica. The fibre content is low in Momordica Chochinchinensis. For reasons already discussed it is not possible to compare correctly the data of this work with other available data.

SUMMARY

1. Three varieties of Momordica, known by the name of Kankrol have been analysed.

2. Botanical name of the third is probably unknown.

3. There are reasons to believe that in certain cases there may be some confusion in proper indentification of the variety of Kankrol analysed.

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ON THE ANTIHAEMOLYTIC ACTION OF CERTAIN SULPHANILAMIDE COMPOUNDS

By

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The antihæmolytic action of the Sulphanilamide compounds has been the subject of some interest during recent years. Levaditi and Vaisman (1935) found that sulphanilamide neutralized the hæmolysins and leucocidins of streptococci. Gross, Copper and Lewis (1938) on the other hand observed that it caused a very slight, almost negligible, inhibition of the action of streptococcal and staphylococcal hæmotoxins. Prontosil (*i*) according to these workers had no inhibitory action on streptococcal hæmolysins, but the inhibition was more in evidence with prontosil (*ii*) and a veriety of other substances of altogether different nature such as eosin, Hgcl2, phenol and Na2CO3. Huntington (1938) and Kemp (1938) similarly found that *in vitro,* in concentrations equal to or greater than those therapeutically induced in the body fluids, sulphanilamide was without apparent effect upon streptococcal hæmolysin.

Roy, Mozumdar and Mukherjee (1940) studied the action of soluseptasine (May & Baker) on some of the common hæmolytic substances such as cobra venom, bile salts, saponins, and cyclamin *in vitro,* and found that it had a definite retarding action on the hæmolysis caused by them. Bacterial hæmolysins such as those elaborated by *vibrio cholerae* and streptococcus hæmotyticus were also found to be neutralized by soluseptasine. In this present paper the action "prontosil" on some of the hæmolysins studied earlier has been discussed, and attempts have been made to throw some light on the mechanism of the antiæemolytic action of these compounds.

Technique:—

The action of prontosil was studied by using the same general technique as that employed in the case of soluseptasine except that the extent of hæmolysis was judged from the amount of residual r.b.c. and not from the colour of the supernatant fluid.

Effect of Prontosil:—on cobra venom hæmolysis.

A 5.0 per cent solution of prontosil (Bayer), 0.1 per cent solution of cobra venom in normal saline and 5.0 per cent suspension of human r.b.c. were used.

No.	c.c. r. b. c.	c.c. Venom Soln.	c.c. N. Saline	c.c. Prontosil.	Time for haemolysis ½ hr.	1 hr.	2 hrs.	21 hrs.
1	.3	.5	..	.2	—	—	—	—
2	.3	.5	.1	.1	—	—	0.2	1.0
3	.3	.5	.15	.05	—	—	1.0	1.0
4	.3	.5	.2	..	—	1.0	1.0	1.0
5	.3	.4	.1	.2	—	—	—	—
6	.3	.4	.2	.1	—	—	0.1	1.0
7	.3	.4	.25	.05	—	—	1.0	1.0
8	.3	.4	.3	..	—	1.0	1.0	1.0
9	.3	.3	.2	.2	—	—	—	—
10	.3	.3	.3	.1	—	—	0.05	1.0
11	.3	.3	.35	.05	—	—	0.3	1.0
12	.3	.3	.4	..	—	1.0	1.0	1.0
13	.3	.2	.3	.2	—	—	—	—
14	.3	.2	.4	.1	—	—	0.05	1.0
15	.3	.2	.45	.05	—	—	0.2	1.0
16	.3	.2	.5	..	—	1.0	0.1	1.0
17	.3	.1	.4	.2	—	—	—	—
18	.3	.1	.5	.1	—	—	0.05	0.1
19	.3	.1	.5	.05	—	—	0.1	1.0
20	.3	.1	.5	..	—	1.0	1.0	1.0
21	.3	..	.5	.2	—	—	—	—
22	.3	..	.6	.1	—	—	—	—
23	.3	..	.65	.05	—	—	—	—
24	.3	..	.7	..	—	—	—	—

1.0 COMPLETE HAEMILYSIS.
NO HAEMOLYSIS. .....

These experiments were repeated several times with almost identical results. It would appear from the above that prontosil retards the haemolytic action of cobra venom in the same manner as does soluseptasine, the former appearing to be more effective of the two in this respect. Prontosil was also found to have a similar anli haemolytic action on some of the other haemolysins studied earlier viz. the bile salts, the saponins and the bacterial haemolysins (El Tor and Streptococcal).

The Mechanism of the antihaemolytic action:

While the mechanism of haemolysis caused by the large majority of lytic substances is not clearly understood and must of course vary with the nature of the haemolysin concerned, it is peculiar that these sulphanilamide compounds should retard the haemolytic action of substances coming under

so many different categories such as snake venoms, saponins, bile salts, glucosides and bacterial haemolysins.

Their effectiveness against such a wide variety of haemolysins would suggest that they act either by exerting a protective action on the red blood cells or by neutralising the haemolysin in some manner. The following experiments were done with a view to gain an insight into the nature of this antihaemolytic action exerted by these substances against cobra venom haemolysis. Since the action of these compounds are somewhat similar, the experiments conducted with one of them (Viz. soluseptasine) will be reported by way of illustration.

TABLE. II

Cobra venom .. .. 0.1% solution.
Solusepatine .. .. 5.0% „
Human r. b. c. .. .. 5.0% suspension.

No.	c.c. r. b. c.	c.c. Lysin.	c.c. N. Saline.	c.c. Solu-septa-sine.	Time for heaveoly gis ½ hr.	Time for haemolysis 1 hr.	2 hrs.	21 hrs.	Remarks.
1.	.3	.5	..	.2	—	0.6	1.0	1.0	Soluseptasine and r. b. c. were
2.	.3	.3	.2	.2	—	—	1.0	1.0	incubated for 2 hrs. at 37°C
3.	.3	.2	.3	.2	—	1.0	1.0	1.0	and then the venom solutions
4.	.3	.1	.4	.2	—	1.0	1.0	1.0	added.
5.	.3	.3	..	.2	—	—	1.0	1.0	Soluseptasine and venom solu-
6.	.3	.3	.2	.2	—	—	1.0	1.0	tion incubated for 2 hrs. and
.7	.3	.2	.3	.2	—	—	1.0	1.0	then r. b. c.
8.	.3	.1	.4	.2	—	—	—	1.0	added.
9.	.3	.5	..	.2	—	—	1.0	1.0	Solutions are added together
10.	.3	.3	.2	.2	—	—	1.0	1.0	without any previous incuba-
11.	.3	.2	.3	.2	—	—	1.0	1.0	tion.
12.	.3	.1	.4	.2	—	—	1.0	1.0	
13.	.3	.5	.2	—	—	0.5	1.0	1.0	Without soluseptasine.
14.	.3	.3	.4	—	—	0.8	1.0	1.0	
15.	.3	.2	.5	—	—	0.0	1.0	1.0	
16.	.3	.1	.6	—	—	0.8	1.0	1.0	

O.2 c.c. of soluseptasine alone produces no haemolysis under the above conditions.

From these figures it appears that soluseptasine acts by neutralizing the venom hemolysin to a certain extent. That it has no protective action on human r. b. c. against cobra venom haemolysis, but has rather a somewhat sensitizing action on them is shown by the first set of experiments where the retarding action of soluseptasine is not manifest and the final result obtained is probably the outcome of two opposing factors (*i*) prolonged contact of soluseptasine with r.b.c. rendering them more fragile and (*ii*) the neutralization of the venom haemolysin causing a retardation of haemolysis. That soluseptasine has no protective action on the r.b.c. but has rather a sensitizing action on them is more prominently brought out by means of the following experiments.

TABLE III.

Human r. b. c. .. .. 5.0% suspension.
Soluseptasine .. .. 5.0% „
Cobra venom .. .. 0.1% solution.

No.	c.c. r. b. c.	c.c. Lysin.	c.c. N. Saline.	c.c. Solu-septa-sine.	½ hr.	1 hr.	2 hrs.	21 hrs.	Remarks.
1.	.3	.4	.1	.2	—	5	5	5	In these experiments the r.b.c. were washed six times with normal saline, then soluseptasine added and incubated for 1 hr. Then the lysin was added and readings taken at stated intervals.
2.	.3	.2	.3	.2	—	—	5	5	
3.	.3	.1	.4	.2	—	—	—	5	
4.	.3	.4	.1	.2	5	5	5	5	R. b c. washed 3 times with normal saline were incubated with soluseptasine for 1 hr. The r.b.c. were again washed with normal saline thrice to get rid of soluseptine. Then the lysin was added and readings taken.
5.	.3	.2	.3	.2	5	5	5	5	
6.	.3	.1	.4	.2	5	5	5	5	
7.	.3	.4	.3	..	—	5	5	5	To r. b. c. washed 6 times with normal saline, the lysin was added and readings were taken.
8.	.3	.2	.5	..	—	5	5	5	
9.	.3	.1	.6	..	—	5	5	5	

From above it is apparant that contact of the r.b.c. with soluseptasine for a certain length of time renders them more susceptible to cobra venom haemolysis, if the corpuscles are washed free of soluseptasine before the addition of venom. If however the cobra venom and soluseptasine are first incubated together (Experiments 5, 6, 7 and 8—Table II) and then the r.b.c. added or the soluseptasine and the r.b.c. are incubated for a certain period and then the cobra venom in the presence to soluseptasine (Expts. 1,2,3, Table III), there is always a retardation. The neutralizing action of soluseptasine on the venom haemolysin more than counterbalances its sensitizing action on the r.b.c. and the net result is a retardation of haemolysis. Experiments with soluseptasine and sodium taurocholate and also prontosil and cobra venom, on the lines indicated above also point to similar mechanism of action of these sulphanilamide compounds.

Animal experiments:

To see if soluseptasine has a neutralizing action on any of the other toxic constituents of the cobra venom, a few experiments were done with mice. In some of the animals cobra venom alone (0.1 c.c. of 10-20 mg. per cent solution in normal saline) was injected subcutaneously and in others, the same dose of cobra venom with soluseptasine (0.1—0.2 c.c. of 0.5 per cent solution in normal saline). These experiments, thought not sufficient in number on which any satisfactory conclusion, could be drawn, mergrable to showed that the animals receiving both cobra venom and soluseptasine died sooner than those receiving cobra venom alone. The confrol animals tolerated the doses of soluseptasine quite well. The significance of these increased toxicity is not apparent.

1. Prontosil like soluseptasine ex... caused by cobra venom, bile salts, and bacte... ... 42
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2. These compounds appear to act in two opposing direction... susceptible to the action of the lysin and (2) they have also a neutralizing action on the preponderance of one or the other of these opposing forces.

3. The results of a few animal experiments, showed that the mice receiving a mixture of cobra venom and soluseptasine died sooner than sooner than those having cobra venom alone.

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The Indian Journal Of Physiology And Allied Sciences

Vol. II JANUARY, 1948 No. 1

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Printed and published by
NIBARAN CHANDRA DAS,
Prabasi Press, Calcutta.

The Indian Journal of Hospital Pharmacy

Vol VI. January to December 1969 Index to Subjects

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JANUARY, 1947

Published by

THE PHYSIOLOGICAL SOCIETY OF INDIA,

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Price Rs. 10 per volume.

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THE INDIAN JOURNAL OF PHYSIOLOGY and ALLIED SCIENCES

FOREWORD

The birth of the Physiological Society of India, which has sponsored the publication of this Journal, is a landmark in the history of the development of the study of physiology in this country. In January, 1934, it was decided at the Annual Session of the Indian Science Congress Association that a National Academy of Sciences (the present National Institute of Sciences of India) would be established with sections representing the various branches of science, but physiology was not included therein. This created a keen feeling of disappointment amongst certain physiologists in Calcutta. It was realised that unless leading workers in Medical Sciences were convinced of the necessity of separation of physiology from the Medical Section at the Science Congress and the physiologists themselves could form a society for the advancement of researches in this subject, physiology could not secure any position in the temple of sciences in India. These considerations were the chief factors in the inauguration of the Physiological Society of India in July, 1934. Within a short time after the inauguration of the Society it was possible for its promoters to secure whole-hearted co-operation from the leading medical men of Calcutta and from the two institutes of research, *viz.*, the School of Tropical Medicine and the Institute of Hygiene and Public Health. Important papers for discussion at the meetings of the Society began to pour in from various quarters. In the meantime, the section of physiology having been opened in the Science Congress, a large number of papers on physiology and allied sciences from various parts of India were discussed in this section. It was soon felt that the papers discussed at the meetings of the Physiological Society of India and the Physiology Section at the Science Congress should be published in our journal, *viz.*, the Indian Journal of Physiology. The Vice-Chancellor of the University of Calcutta was, therefore, approached for kindly providing for facilities of printing and publishing our Journal from the University Press, but on account of heavy

work of the press and its limited capacity, it was not possible to do it. Vigorous attempts were then made to get donations from sympathetic Princes and from an eminent physician of Calcutta with no better results. Col. S. L. Bhatia of Bombay was at this stage requested to gather opinions from the leading biochemists, medicalmen and physiologists of different parts of India for the publication of a combined Journal of Physiology and Biochemistry, so that funds could be obtained from various parts of India. It was found that the opinions were sharply divided on the question of a joint publication.

How difficult it is to get money for a good and unostentatious cause and to promote it! Having failed repeatedly in this way, the enthusiasm of the promoters languished for a while, when Prof. A. V. Hill came to this country in 1944, our hopes were kindled and it was possible to get a donation of Rs. 1,000 from the late Sir U. N. Brahmachari for our noble cause. His charity has really paved the way for the publication of this Journal. We hope and believe that once the Journal has seen the light of day, it will never again stray into darkness. We are genuinely anxious to enlist the sympathy of all the interested men of this country for the advancement of our cause and wholeheartedly welcome their co-operation, active support and suggestions for the prosperity of this Journal.

Prof. A. V. Hill has very kindly encouraged us in our venture. We take the liberty of reproducing in detail his letter of encouragement to us. We also hope to be pardoned for reproducing in the first issue the photos of two of the eminent persons who were associated with our endeavour and to whom we are deeply grateful for their active support and sympathy with the prosperity of our society.

90, Park Street,
Calcutta,
1st July, 1946.

On the auspicious occasion of the advent of this Journal I have been invited to write a few lines in way of pronouncing benediction on the new born. Is it "Physiology"? Need I say that the heart of this octogenarian beats faster and I feel younger at the very mention of that magic word?

Memory takes me back to nearly half a century ago, when I was called upon to do some spade work in the cause of Physiology. How fascinating that call was! The seed was sown and then followed patient tending and watching. To the outsider the time was long and uneventful. But steadily—though unobtrusively—wonderful developments proceeded, culminating in the fulfilment of the promise in a manner that gave the impression of a good harvest.

To-day, the position of Physiology as an independent science of paramount importance has been assured. The help of Physiology has been sought for by the world in times of war and peace alike. It has come to the front conspicuously as a science of great national value, not merely as an ancillary to Medicine but even more effectively in its service to man's daily life.

The new Journal is the organ of the Physiological Society of India, which was inaugurated a few years ago, mainly through the indefatigable efforts of a band of my valued colleagues (my former pupils). The Society is not yet out of its teens, but even during its infancy it achieved wonders and challenged the admiration of its rivals. Through the medium of the Journal the Society seeks to give publicity to, and broadcast, the activities of Physiologists in India and of researches in kindred subjects. This highly commendable enterprise speaks for itself and I have no manner of doubt that it will receive generous support of those interested in the subjects concerned.

In the closing days of my life, when the flesh is weak although the spirit is most willing and I am denied active participation in the work that has been nearest and dearest to my heart, I rejoice to find that the work of dissemination of Physiology is continued and greatly enhanced by my former pupils and loyal disciples, in various seats of learning in India. May they staunchly hold aloft and keep flying the banner of Physiology, wherever they be.

On the publication of this Journal my joy knows no bound. It is glorious to think that I have lived to see the materialization of my long-cherished desire. I welcome the august guest most warmly and wish it full measure of success.

S. C. MAHALANABIS

THE ROYAL SOCIETY,
Burlington House, London W. 1,
Regent 3335, Sec. B/St.
10 October, 1945

Dear Sir,

As an honorary member of the Physiological Society of India I was delighted to hear of your plan to found your own journal *The Indian Journal of Physiology and Allied Sciences*. I am sure that this journal will play a valuable part in encouraging the development of these sciences in India.

Having myself, for a period of years, been an editor of *The Journal of Physiology*, and having since then been responsible for the biological *Transactions* and *Proceedings* of the Royal Society, I know that editorship is not always an easy task. Long experience, however, has shown me how much willing help is available from one's colleagues in acting as referees and in giving expert advice and I am sure that by co-operation of this kind the Physiological Society of India will be able to maintain a high standard in what is published in the new journal. By setting and maintaining that high standard the *Indian Journal of Physiology and Allied Sciences* will be able to do a real service both to science and to India.

With kindest regards,

Yours sincerely,
A. V. HILL

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Prof. S. C. Mahalanabis

Late Sir U. N. Brahmachari

The Indian Journal of
Physiology and Allied Sciences
Vol. I, No. 1, 1947

THE EFFECT OF CARDIAC DRUGS ON HEART TISSUE EXPLANTED IN VITRO

By N. N. DAS

(Department of Physiology, University College of Science, Calcutta University)

Introduction

In the history of medicine, remedies have often been used for therapeutic purposes on the basis of clinical and empirical experience without a detailed knowledge of the actual physiological processes leading to their specific effects. Although the stimulating and depressing effect of certain drugs on cardiac tissue has been known since the beginning of medicine, enough experimental data were not available to show whether these drugs act directly on the heart through circulation or indirectly through the nervous system. The knowledge of the true nature and the exact site of action of a drug on the heart is of some importance both to pharmacologists and physiologists. It is not enough in modern scientific medicine to know, for example, only the effect of a drug on the rate of the heart though such knowledge may serve the purpose of the clinician. It is necessary to know whether this change in the heart rate is brought about through an action on the ganglion cells, the nerve-endings, the neuro-muscular substance, or the heart muscle proper. Upon such knowledge depends the advance of medicine in its theoretical and practical aspects. The development of the newer technique of cultivating embryonic tissue outside the body has given the modern medical scientists a very important tool for solving many of their difficulties in connection with the nature of action of drugs on the heart, which could not be satisfactorily answered before.

Short History of Tissue Culture and Its Technique

The technique of tissue culture has generally been considered somewhat forbidding, but its underlying principles are really simple and can be readily comprehended. It consists of removing tissues from the body under sterile conditions and incubating them in specially prepared media conducive to their growth. This method has been successfully used with tissues from a great variety of organisms, both vertebrate and invertebrate, and also has been applied to plant tissues.

Tissue culture is generally dated from 1907 when Harrison first cultivated nervous tissues from frog embryos in frog lymph. It should be noted, however, that the possibility of growing tissues outside the organism had

already been demonstrated by Leo Loeb in 1897, when he grew tissues in blood plasma in test tubes. Loeb's method is still used with good result by some investigators, *e.g.*, Champy (1913) when it is desired to fix the culture for serial sections. Harrison's method is better adapted to the examination of the living culture under the microscope. Burrows, stimulated by the work of Harrison, developed a method for growing chick embryonic tissues in vitro and in 1910, with the co-operation of Carrel, laid the foundations of the present solid medium technique, when they grew adult tissues in blood plasma (Burrows, 1913).

A considerable advance was made in the technique when Carrel discovered (1912) that, while coagulated plasma makes an excellent framework for the cells in the culture it does not contain the substances necessary for their continued growth. He found that the addition of aqueous extracts of embryos renders indefinite proliferation possible. An evidence of this is given by his strain of fibroblasts which has already lasted twenty-one years. The life of cells in cultures is now limited only by the care taken to keep them free from bacterial contamination and by technical skill in transplanting them.

In its essentials, the method used today is that elaborated by Carrel. A fragment of tissue is embedded on a cover slip in a drop composed of plasma and embryonic extract. The cover slip is mounted and sealed over a depression slide. Two to three days later the original fragment, surrounded by its new growth, is cut in two and each piece transferred to a new medium. This procedure can be kept up indefinitely.

A more recent way, which is less disturbing to the tissue but not so adaptable to high power microscopic observation, is the use of small pyres, so-called Carrel flask (1923) of various types. In these, the tissue is embedded in a plasma clot with a supernatant nutrient fluid medium; and if the supernatant fluid is changed every few days, the tissue, if it is slow growing such as epithelium, may remain undisturbed for several weeks. In the case of rapidly growing fibroblasts, the fragments of tissue must be transplanted about every ten days.

Maximow (1925) introduced an excellent method which permits the washing and feeding of the cultures on cover slips without disturbing the tissues. A small circular cover slip is fastened by a thin film of Ringer solution to the centre of a larger square cover slip and the tissue is embedded in a plasma clot on the circular cover slip. The square, with its adherent circle, is then mounted and sealed over a large depression slide. Every few days the circular cover slip with the tissue on it is removed asceptically from the square cover slip. It is immersed in a Tyrode bath for about ten minutes, mounted on a clean square, fresh medium added, and sealed as before.

In the Strangeways Laboratory (1926) at Cambridge, England. small watch crystals are used as containers for cultures for embryological studies; the crystals, surrounded by absorbent cotton, are sterilized in Petri dishes; the tissue is placed in a clot of plasma and extract in the crystal, the cotton is moistened, and the Petri dish is covered and sealed with vaseline.

The fluid medium technique was developed by Lewis and Lewis (1911) at Johns Hopkins University and it is used by many investigators. This method

is remarkably simple and consists of placing a fragment of tissue in a hanging drop. This drop may consist of sea water, Ringer or modifications of it, serum, embryo extract, or mixtures of these. The cultures can survive for several days, and the thin sheets of outgrowing cells make excellent preparations for histological and cytological studies. Growth is not, however, extensive and subcultures cannot be made with any assurance of success.

This technique (Jane Hogue, 1937) was employed to detect the development of myo-fibrillae often with optical segmentation in heart explants cultivated in a special liquid medium (vide Fig. 1). Murray (1932) investigated the effect of different ions on the character of fibrillation and co-ordination of throbbing of heart explants. The knowledge of tissue culture technique advanced by all these studies has been used by me for the specific purpose of testing pharmacological action of drugs on heart explants.

Fig. 1
Tissue cultures of heart muscle cells
(a) Photomicrograph of cardiac muscle from 43-day culture, showing binucleate cells and wavy (A) and straight myfibrillae, x530.
(b) Photomicrograph of cardiac muscle cells at the edge of 48-day cultures x530.

Materials and Method

The following procedure was adopted in preparing the tissue explants used in my experiments:

(*A.*) *Experimental Set-up.*

The experimental work was performed in the cool room which maintains a more or less uniform temperature and is free from dust. This was important for the success of the experiments. To ensure additional cleanliness and absolute freedom from dust particles a glass slab was placed on the working table and it was wiped with cotton swabs moistened with alcohol. Fresh sterile test-tubes were placed underneath pipettes kept in a pipette rack and fitted up with nipples. To facilitate the work a regular order of these tubes with their pipettes should be maintained throughout the experiment. Thus the first tube may hold the chicken plasma, the second, with the embryo extract pipette, then two or three, tubes with empty pipettes for different experimental substances; and the last tube to hold Pannet's solution (to be described later) pipette of heavier calibre.

The box of depression slides, cover slips, Petri dishes (to be used as covers), instruments, vascline, etc., were then removed from their paper wrappings and arranged in a convenient position on the culture table. All of them were kept covered except when an article was removed for immediate use.

A previously prepared bowl of cracked ice was placed on the assistant's table. Tubes of the required medium from the refrigerator were removed and allowed to stand in an upright position in a bowl of ice containing chicken plasma, chick embryo extract, and anything that might be required. Small quantities of these were stored in numerous separate tubes in order that fresh ones might be used frequently. In this way, any break in technique did not jeopardize the entire supply.

The same precaution was taken with Pannet's solution. Its composition is given below..

Stock Solution A.

Sodium chloride NaCl	12.11 g.
Potassium chloride KCl	1.55 g.
Calcium chloride $CaCl_2$	0.77 g.
Magnesium chloride $MgCl_26H_2O$	1.27 g.
Water	100.00 c.c.

Stock Solution B.

M/69 sodium dihydrogen phosphate $NaH_2PO_4H_2O$	5 c.c.
M/69 disodium hydrogen phosphate $Na_2HPO_4.12H_2O$	55 c.c.

Solutions A and B are mixed in proper proportions to make the Pannet's solution.

The neck of the paraffined flask containing the stock supply of Pannet's solution was flamed and from it 50 or 75 c.c. was poured out into a 125 c.c. Erlenmeyer flask. The pH of the Pannet's solution was to be checked at this time since it is liable to change. It should be between 7.6—7.8.

Tissues will survive within a pH range of 7.0 to 8.0 with the optimum growth at the pH of the homologous plasma. Chicken plasma has a pH of 7.8 to 8.0; and mammalian, 7.4 to 7.6. The same Pannet's solution was used with both, since it quickly assumed the pH of the more highly buffered plasma when mixed with it.

Phenol red is a very useful pH indicator for tissue cultures, as it has a range of pH from 7.0 to 8.2, is not altered when sterilized by heat, and is relatively nontoxic. A quantity sufficient to indicate the pH may be added to any culture medium without injury to the cells. In this laboratory, we sterilize about 1 c.c. of 0.5 percent aqueous solution in ampules and add one or two drops of this to about 1 c.c. of the embryonic extract. This method obviates frequent resterlization of the phenol red, which we find renders it slightly toxic. Another good method of sterilizing phenol red is to filter it through a Berkefeld filter.

The granite cup containing the wax which was to be used for sealing the cultures was then put over a low Bunsen flame or on an electric plate. A trace of vaseline added to the paraffin prevented it from chipping. Parawax, already prepared, was convenient.

(B.) Preparation of tissues.

If adult tissues are to be used the danger of infection must be considered and care must be taken to retract the skin and to avoid puncturing the alimentary canal when making dissections. If it is not possible to prepare the cultures as soon as the tissue is excised, the tissue should be covered with Pannet's solution in a tightly stoppered vessel and stored in the refrigerator until used. We have cultivated tissues successfully even after forty-eight or seventy-two hours in cold storage, but this is an emergency measure and not recommended for best results.

To excise a fragment of the tissue to be cultivated a sharp sterile knife was used, avoiding unnecessary pressure of tearing. The fragment was then placed in an oval depression slide containing Pannet's solution. If blood is present it should be washed several times until the fluid is absolutely colourless. The fragment was then held with a platinum needle on the flat platform of the depression slide and cut into pieces 1 to 2 mm. in size with a sharp cataract knife; a dull one would tear the cells. (Some workers use scissors for cutting the tissues because it is quicker, but difficulty is encountered in obtaining pieces of uniform size, and there is apt to be more cell injury). The pieces or fragments were then returned into the deep part of the depression slide where they were covered with Pannet's solution and finally covered with a Petri dish.

(C.) Mountings of embryonic chick heart explants.

The following technique, which is a modification of Carrel's method (Chopra, Das, Mukherji, 1936), was adopted by me during the experiment. The hearts were removed aseptically from embryonic chicks of various ages (2 to 7 days) and were cut into fragments of about 1 sq. mm. in area. It was usually possible to do this with the naked eye; a dissecting microscope was seldom used in our series as very small bits of heart tissue which were cultivated never survived for a sufficiently long time. The heart fragments were then suspended in a mixture of extracts derived from the embryo and of the homologous plasma obtained from a cock. Two sets of slides were prepared with a view to watch the two different characteristics of the heart explants at their best, namely, (1) the throbbing of the heart and (2) the fibroblastic growth of the tissue cells. In the first set of slides where the rate of throbbing was the important result to be observed, embryo extract was avoided as far as possible, but a drop of extract was necessary to help in the clotting of the hanging drop. Under such conditions the throbbing went on for a number of days (8 to 14 days) without any change of medium. In the second set of slides where the idea was to watch the rate of tissue growth only, the explants were prepared with liberal quantities of embryonic extract which apparently possessed a stimulating effect on tissue growth.

To study the effect of cardiac drugs, both types of explants were treated with various dilutions of the drugs under investigation, and controls were kept side by side for purposes of comparison. More than a dozen explants were made in each case and every dilution was repeated at least six times. The slides were kept in an incubator at 37.5°C, and examined every day inside a microscope incubator to record the rate of throbbing. The relative growth of tissues in the second series of slides was also measured daily with the help of camera lucida on graph paper. The number of squares covered by a particular explant was calculated and the percentage increment from day to day was recorded and comparisons made with the controls of the same series. Usually, records were maintained for 8 to 9 days but, in a few cases, longer periods of observation were necessary.

Advantages of Tissue Culture Technique in the Present Study

Pharmacologists and physiologists have long attempted to analyse and locate the seat of action of cardiac drugs by a variety of experimental methods involving the use of 'denervated' or isolated hearts or by utilizing the selective affinity of certain chemical agents to produce depression or paralysis of ganglion cells and nerve-endings. The dual nature of the nerve supply and other complexities associated with cardiac innervation however offer considerable difficulties in the way of investigation and most of the data in this field are largely inferential and tentative. Thus, it is almost impossible to denervate completely the heart of adult vertebrates to test the action of drugs on the muscle. If the cervical and thoracic sympathetic chains are removed as has been done by Cannon et al (1929), the sympathetic nerves of the heart degener-

ate completely and no regeneration is possible as the cell bodies have been removed. Cardiac vagus nerve, unlike the sympathetic, cannot, however, be entirely eliminated, as even after complete section of the vagus, the post-ganglionic fibres have been known to survive (Cameron, 1933) and therefore one can draw no safe conclusions regarding the site of action of drugs by using the so-called 'denervated' hearts. By using an isolated heart, one may be sure that the effects on such a preparation must be of peripheral origin but this does not exclude the possibility that the action may be either through the intrinsic nerve endings or the neuromuscular substance of the heart or directly on the cardiac muscle. By the judicious use of chemical agents like nicotine, atropine and ergotoxine, very useful information regarding the point of attack of different cardiac remedies may be obtained but even these data are not always conclusive.

It was found that embryo's of vertibrate heart in earlier stages of development are free from nerve. The automatic rhythm of heart muscle develops before the nerves enter the muscle. This type of aneural heart tissue of embryo is the suitable material for studying the direct action of drugs on the muscle tissue of the heart.

Results—

The following drugs have been tested—

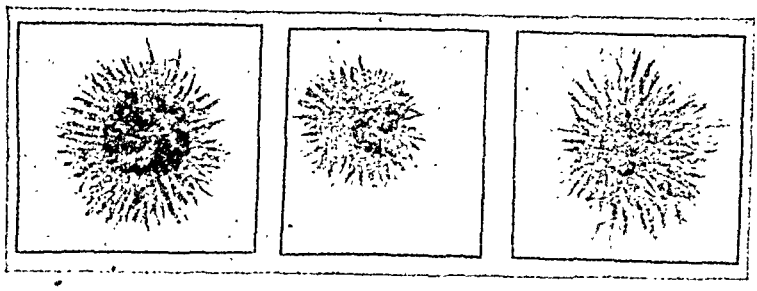
(1) Digitalin (pure glucoside)
(2) Nerifolin (pure glucoside T nerifolia)
(3) Cactus (active principles of Cactus Grandiflora)
(4) Arjun (active principle of Terminalia Arjuna)
(5) Gulbanafsa (active principles of Viola Odorata)
(6) Camphor

The drugs were collected from reliable sources. The preparation of solutions were done aseptically. The solutions were ultimately passed through L_2 candles for perfect sterilization. The stock solutions of particular strength thus prepared were stored in a refrigerator and diluted with Pannet Comptons (1924) isotonic solution for the use of tissue culture.

The two different aspects of this tissue culture were taken into account

(1) The rate of throbbing after 36 hours' incubation.
(2) On the rate of tissue growth after three days' incubation.

The table I and Figs. 2 & 3 shows graphically the results. The explants were very carefully treated during the whole course of experiment, so that they can be kept alive for a longer period. The table shows that the first three drugs, namely, Digitalin, Nerifolin, Cactus have depicted results which are of similar nature, of course, there are difference in the quality of response. Digitalin in dilution 1 in 10,000 to 1 in 50,000 produces a gradual lowering in the rate of pulsation. The rate of growth of explants is retarded in these dilutions. As more concentrated drugs were used 1 in 1000 to 1 in 100 the toxic effect was more marked and was shown by stoppage of throbbing and fatty degeneration of the tissue explants. When the explants were treated with higher dilutions alone 1 in 50,000 the inhibitory action is feebly marked. The rate of throbbing is



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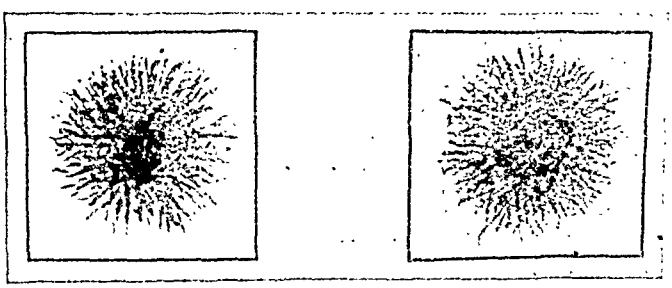

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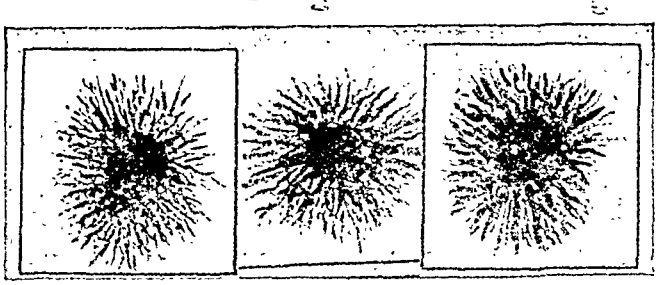

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Fig. 3

Figure 3. Camera-lucida drawings of the growth of cardiac explants.
(a) Control explant. (b) Digitalin (1 in 40,000) showing retardation of growth. (c) Digitalin (1 in 100,000) showing slightly greater growth than control. (d) Nerifolin (1 in 100,000) slight stimulation of growth. (e) Cactus (1 in 100.000) slightly greater growth than control but less than Nerifolin. (f) Gulbanafsa (1 in 10,000). (g) Arjuna (1 in 10,000). (h) Camphor (1 in 5,000).

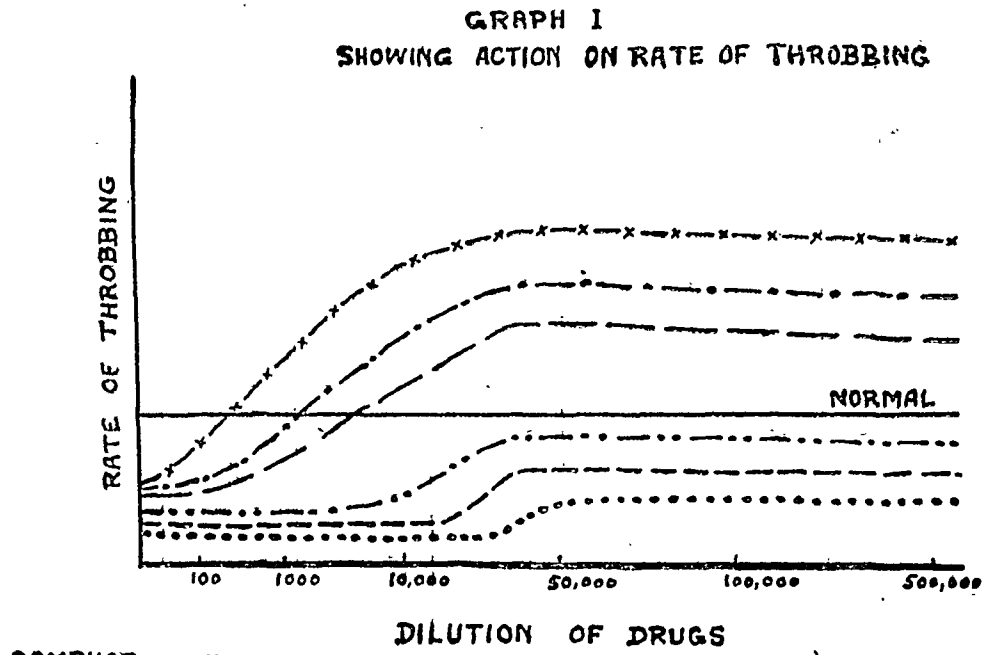
TABLE I.

Effect of cardiac drugs on heart explanted in vitro.

Dilution.	On the rate of throbbing (after 36 hours).						On the tissue growth (after 72 hours).					
	100	1,000	10,000	50,000	100,000	500,000	100	1,000	10,000	50,000	100,000	500,000
Digitalin ...	Stop	Stop	Stop	Slow	Slow	Slow	*Nil*	*Nil*	*Nil*	Less than control —	Same as control =	Enhanced +
Nerifolin ...	Stop	Stop	Stop	Slow	Slow	Slow	*Nil*	*Nil*	Less than control —	Less than control —	Enhanced +	Enhanced + +
Cactus ...	Stop	Stop	Stop	Slow	Slow	Enhanced	*Nil*	*Nil*	Less than control —	Less than control —	Same as control =	Enhanced + +
Arjuna ...	Stop	Slow; enhanced later.	Enhanced	Enhanced	Enhanced	Enhanced	*Nil*	Less than control —	Less than control —	Enhanced +	Enhanced + +	Enhanced + + +
Gulbanafsa ...	Slow; enhanced later.	Enhanced	Enhanced	Enhanced	Enhanced	Enhanced	*Nil*	Less than control —	Same as control =	Enhanced +	Enhanced + +	Enhanced + + +
Camphor ...	Slow; enhanced later.	Enhanced slightly.	Enhanced	Enhanced	Enhanced	Enhanced	Slightly less than control =	Less than control —	Enhanced +	Enhanced + +	Enhanced + +	Enhanced + + + +

N. B.— + means approximately 25 per cent increment of fibroblastic out-growth compared to normal controls.
++ means 50 per cent. = indicates doubtful increase.
+++ 75 per cent and so on. — indicates negative increase (less than control).

lower than normal, the pulsations are however more powerful and long lasting. A method of measuring the time interval between the two beats and the period of contraction is devised by connecting two fine platinum electrodes at the two



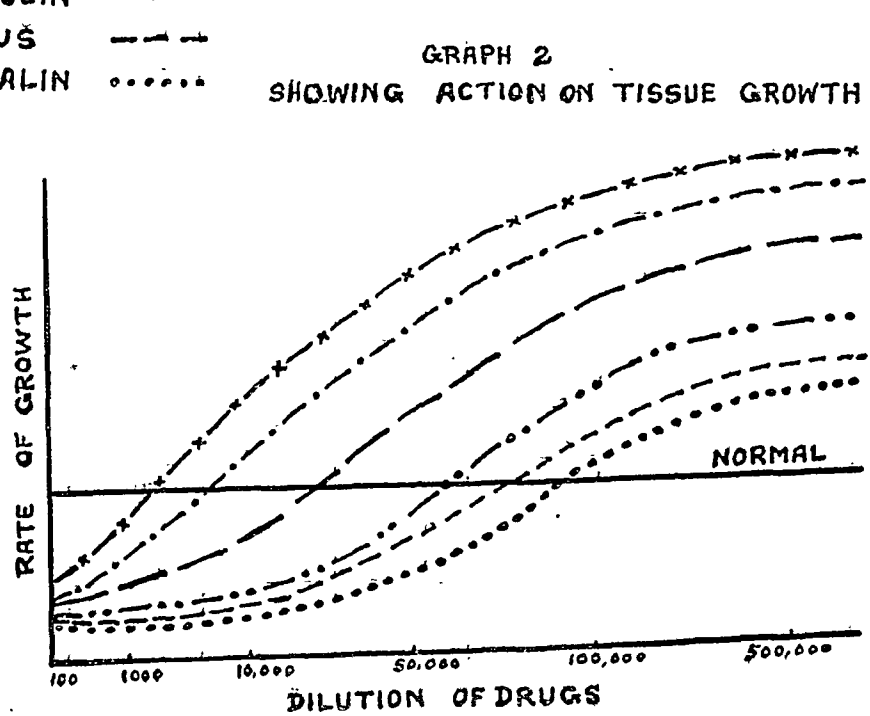


Fig. 2

opposite points in the explant and connecting them with the oscillograph (Chopra and Das 1938). This gives a definite idea about the time relation of the rate of throbbing. The tissue growth is also increased in these higher dilutions like 1 in 100,000. Nerifolin and Cactus are slightly weaker than Digitalin, the diminishing effect on the rate of throbbing in these cases is just disappearing near about the dilution of 1 in 10,000. The tissue growth retardation is less prominent than in digitalin (see Fig. 2). Arjun, Gulbanafsa and Camphor have a contrasty action with those of the first three drugs. But in highly concentrated condition like 1 in 1000 the rate of throbbing gradually increase and are forceful. The explants grow in a rapid rate and in a short time almost whole of the cover-slip is filled with new growth. The explants have a longer life and tonic condition of throbbing. The growing cells are also resistant to fatty degeneration unlike the previous ones. Camphor has more powerful action than Arjun and Gulbanafsa. But Arjun shows a singular tonic condition of the explanted tissues which gives a lasting effect on the rate of throbbing.

Discussion—

The results show certain peculiar aspects of the cardiac drugs. The first three drugs, *viz.*, Digitalin, Nerifolin & Cactus have more inhibitory action, on the other hand, Arjun, Gulbanafsa and Camphor have a tonic and stimulating effect on heart explants. The action of Digitalis is ascribed as a vagomiometic drug and changes in the heart's action is said to be due to central and peripheral vagal stimulation, lowering the tone of the cardiac muscle, and rate of conduction through the Purkinje fibres. In the experiments with heart explants it is found that the action of the drug is directly on the muscle tissue. The rate of throbbing is lowered in all the three drugs and the refractory period increased. It is well known from the experiment of Lewis (1925) that after a prolonged administration of digitalis the T-wave of the Electrocardiogram is inverted showing thereby that Digitalis group of drug behave more as hypnotic than stimulant on the heart. The other three drugs Arjun, Gulbanafsa and Camphor which have been used in the indigenous system of medicine from days of yore are supposed to produce beneficial effects on the heart. In experiments on the heart explants in fairly concentrated dilution they have a marked stimulating effect like caffein without producing any toxic action.

Camphor is also used is all countries of this world as a stimulant of the failing heart. It is a powerful stimulant to the central nervous system. From the tissue culture experiments it is found that it has also a direct action on heart muscle.

Summary and Conclusions

A number of cardiac remedies in common use have been studied on the throbbing aneural embryonic heart explants cultivated in slide coverslip preparations. Provided sufficient precautions are taken in the experimental technique, this method can be satisfactorily used for the study of the site of action of drugs which cannot be done by the ordinary methods at the disposal of Physiologists and Pharmacologists. This method further gives the promise of being useful in a number of other Physiological & Pharmacological studies.

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The Indian Journal of
Physiology and Allied Sciences
Vol. I, No. 1, 1947

EXPERIMENTAL STUDIES ON THE INFLUENCE OF THIAMIN (VITAMIN B_1) ON THE INFLOW OF PHOSPHATES INTO, AND ABSORPTION OF GLUCOSE FROM, THE INTESTINAL CANAL.

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The influence of thiamin on the maintenance of the tone of the alimentary canal and of its normal functioning is well known (Cowgill-1939). It may, therefore, help indirectly the absorption of products of digestion from the intestine, but in view of its immense importance to carbohydrate metabolism it is considered that it may help specifically the absorption of glucose from the intestine. Experiments were, therefore devised and carried out to ascertain how far this assumption is correct.

Experimental

Rabbits having usually a fairly long jejunum, were selected for experiments about absorption. They were starved for 24 hours and then anaesthetised by injection with urethane solution (1.5 mg per kg of body weight). After laparatomy was done, 20 cc of warm Tyrode solution (at 38.C°) without phosphate and glucose, was injected with a serum syringe into the intestine just below the pyloro-duodenal junction and a ligature was tied immediately beneath the site of injection. In order that the intestine might be washed of all the adherent constituents, the injected Tyrode solution was slowly moved down the intestinal canal by a mild pressure with fingers. A second ligature was then tied round the intestine 15 c.m. down the stomach to exclude duodenum and a 3rd ligature 30 c.m. below the 2nd, the Tyrode solution having been previously completely removed from the ligated portion. Two other ligatures were then applied, one 2 c.m. below the 3rd and another 30 c.m. beyond the last, (*i.e.*, the 4th ligature,), the Tyrode solution having been removed as before from the ligated part. In consequence of these ligatures two loops each 30 c.m. long were formed, the former being called the upper loop and the latter the lower loop.

Into one of these loops 6 c.c. of hypertonic glucose solution containing 0.324g of glucose (5.4%) and into the other the same amount of this solution containing in addition 10 ug or more of vitamin B_1 were injected, the order of injection of these solutions into these loops being reversed in the

next experiment with a view to find out if there was any difference in the rate of absorption from these loops. Immediately after each injection its site was plugged with collodion-soaked cotton to prevent any loss of fluid therefrom.

Twenty minutes after the injection 1 c.c. of fluid was taken from each loop and was made up to a definite volume with water. It was then divided into two equal portions, one of which was used for free inorganic phosphate estimation by Younberg's colorimetric method after the protein was precipitated by tri-chloracetic acid and the other for glucose estimation by Benedict's micro-method after the removal of protein by Folin-Wu's method with sodium tungstate and sulphuric acid.

One hour after the injection these two loops were cut off and the fluid was drained out from each loop which was then washed with jets of water with a syringe. The drained fluid and the washing from each loop were then collected and made up to a definite volume which was then divided as above into two portions for phosphate and glucose estimation as in the previous experiment.

In some experiments 6 c.c. Tyrode solution was injected into one loop and into other Tyrode solution plus vitamin B_1 in varying amounts, or Tyrode solution plus vitamin B_1 in different amounts in both the loops, as indicated in Tables.

Results & Discussion

The results of these experiments are given in Tables I, II, and III. Table I gives the inflow of phosphates into intestinal loops containing Tyrode solution (phosphate and sugar free) with or without vitamin B_1. It is observed that the amount of phosphate in 1 c.c. of the fluid in a loop 20 minutes after the introduction of Tyrode solution (phosphate and sugar-free) with or without vitamin B_1 does not vary much with animals of widely different weights, the range being between 39.2 ug and 67.0 ug but it varies widely in the whole of the fluid left in the loop after an hour, the range being between 68.4 ug. and 340.0 ug. In the experiments from (1) to (5) there was Tyrode solution alone in one loop and Tyrode plus vitamin B_1 in the consecutive loop. In two of them viz. (3) and (5) there was a greater amount of phosphate in the loop containing Tyrode solution only than in the loop containing Tyrode plus vitamin B_1 but in (1) the reverse was found, and in (2) and (4) the amount of phosphate in 2 loops was nearly the same. In experiments (6) and (7) there were Tyrode and vitamin B_1 in each loop, but the amount of vitamin B_1 was greater in one loop than in the other. In one of them, viz., (6) there was a greater amount of phosphate in the loop cantaining a smaller amount of vitamin B_1, whereas in experiment (7) the amount of phosphate was the same in both loops. It is evident from these experiments that no significant conclusions can be deduced as to the effect of vitamin B_1 on the inflow of phosphates into the intestinal canal within 20 minutes after the introduction of Tyrode solution with or without vit. B_1. The variations in the amount of phosphate in consecutive loops after 20 minutes in several experiments might be explained in the following way. These experiments show that phosphate flows into the intestinal canal containing Tyrode (free from sugar and phosphate) with or without vit. B_1. As this inflow of phosphate is ap-

parently due to diffusion from blood, which is a slow process, it is likely that there would be a difference in its concentration in different parts of the contained fluid, the concentration being greater in that portion of the fluid which is nearer the wall of the intestine than in that which lies in the middle of it. Unless precautions are taken to tap the fluid from the same part of the intestinal content, there would be anomalous variation of phosphate in the tapped fluid.

TABLE I

Showing the inflow of phosphate in two consecutive loops of the intestine containing Tyrode solution in one loop and varying amounts of vitamin B_1 in Tyrode solution in the other loop or different amounts of vitamin B_1 in Tyrode solution in both loops. (Tyrode solution was free from sugar and phosphate.)

No.	Weight of animals	Contents of two loops (6 c.c. of Phosphate & sugar-free Tyrode present in each loop)	*Results after 20 mts.* Phosphate present in 1 c.c. of the fluid taken out of the intestine and expressed in ug	*Results after 1 hr.* Phosphate present in the remaining fluid obtain from the intestinal loops and expressed in ug	Remarks
1.	590. g.	Upper—Plain	45·21	100·00	
		Lower—10 ug B_1	54·86	153·60	
2.	930. g.	Upper—10 ug B_1	53·60	176·10	
		Lower—Plain	53·40	68·40	
3.	940	Upper—15 ug Vit. B_1	58·0	100·7	Profuse bleeding inside the lower loop.
		Lower—Plain	67·0	297·0	
4.	870	Upper—20 ug B_1	46·7	87·7	
		Lower—Plain	45·5	109·7	
5.	640	Upper—50 ug B_1	43·5	89·5	
		Lower—Plain	63·3	271·0	
6.	830. g.	Upper—10 ug B_1	61·40	340·00	
		Lower—20 ug B_1	39·20	300·00	
7.	810. g.	Upper—20 ug B_1	43·70	280·00	
		Lower—40 ug B_1	43·80	179·90	

The results of estimation of phosphate in the residual fluid of intestinal loops one hour after the introduction of modified Tyrode with or without vit. B_1 show the following :

(a) The phosphate content in the loops of different animals may widely vary. This might be due to a difference in the phosphate content of blood in different animals or to a difference in the nature of the membraneous wall of the gut causing a higher or lower rate of diffusion.

(b) In (1) and (2) the loops having 10 ug vit. B_1 contain an appreciably larger amount of phosphate than those without vit. B_1. The experiment (3) is spoilt on account of bleeding inside one of the loops. In (4) (5), (6) and (7) the loops having a larger amount of vit. B_1 contain paradoxically a smaller amount of phosphate than those having either a smaller amount of vit. B_1 or none at all. This paradoxical diminution in phosphate content has taken place in loops having vit. B_1 greater than 10 ug. This can be explained by the supposition that when vit. B_1 is present in a loop in concentration over 10 ug (which may be called its critical concentration) it combines with phosphate to form thiamin-pyrophosphate. As thiamin-pyrophosphate is not estimated by Younberg's method, the loop in which this substance is formed, shows a paradoxical diminution of phosphate content. If vit. B_1 present in each of two loops be in excess of 10 ug, then tiamin pyrophosphate would be formed in both loops, but in greater amount in that loop which contains a comparatively greater amount of vit. B_1. Accordingly, Younberg's method would show a smaller amount of phosphate in the latter loop. As the assumption made above appears to be theoretically plausible and explains the apparent diminution in the phosphate content of loops containing a larger amount of vit. B_1 than 10 ug, the diminution being relatively greater in loops containing much higher amounts of vit B_1, it may be concluded that vit. B_1 introduced into the intestine promotes the inflow of phosphates therein.

Table II records the influence of glucose alone and of glucose with varying amounts of vitamin B_1 on the inflow of phosphates into the intestinal loops and the absorption of glucose from these loops. Of the ten experiments performed five were done with 10 ug vitamin B_1 three with 15 ug, one with 20 ug and one with 50 ug vitamin B_1. The results of estimation of phosphate and glucose in 1 cc of the fluid collected 20 minutes after its introduction show the following:—

1. That the phosphate content in 1cc of fluid collected after 20 minutes is greater, as is expected, in loops containing vit. B_1 in experiments (1), (2), (3), (5), (6), (8) and (10). In experiments (4), (7) and (9) there is a greater amount of phosphate in loops without vitamin B_1 than in loops with it. At first sight this appears to be anomalous, but if the relative amounts of glucose present in the two consecutive loops in these experiments be considered, the apparent anomaly can be explained away. It is known that glucose is phosphorylated before absorption, so that the greater is the absorption of glucose from a loop, the greater is the removal of phosphate therefrom. It will be noticed that the difference in the amounts of glucose after 20 minutes in the 2 loops in experiments (4), (7) and (9) is relatively greater than in other experiments (excepting experiment 6),

TABLE II.

Showing the influence of glucose and glucose plus Vitamin B_1 on the inflow of phosphates in the intestinal loops and the absorption of glucose therefrom.

No.	Weight of animals	*Contents of loops.* 6 c.c. of 5·4% glucose soln. was present in each loop)	Results after 20 minutes. Phosphate present in 1 c.c. of the fluid taken out of the intestine & expressed in ug.	Glucose present in 1 c.c. of the fluid taken out of the intestine & expressed in mg.	Results after 1 hour. Phosphate present in the remaining fluid of the intestine & expressed in ug.	Glucose absorbed* expressed in mg.	Remarks
1.	930 g.	Upper—10 ug. B_1	198	40	390	192	The amount of sugar present in 6 c.c. of 5·4% glucose solution is 0·324 g (324 mg)
		Lower—glucose soln. only	150	44	260	175	
2.	830 g.	Upper—glucose soln. only	200	46·8	247	161·4	
		Lower—10 ug B_1	220	41	360	192·0	
3.	580 g.	Upper—glucose soln. only	50	50	100	160	
		Lower—10 ug B_1	80	49·5	120	188	
4.	720 g.	Upper—10 ug B_1	150	80	217	250	
		Lower—glucose soln. only	220	100	200	170	
5.	1010 g.	Upper—10 ug B_1	169	45·5	360	140	
		Lower—glucose	160	52	400	155	
6.	910 g.	Upper—glucose soln. only	43·2	27·5	109·7	174·3	
		Lower—15 ug vit. B_1	49·3	40·0	97·53	150·0	
7.	890 g.	Upper—glucose soln. only	60·0	36·6	81·0	198·7	
		Lower—15 ug vit. B_1	54·8	27·5	131·0	224·6	
8.	660	Upper—glucose soln. only	23·0	40·74	136·0	99·93	
		Lower—15 ug vit. B_1	29·6	47·82	72·0	191·57	
9.	820	Upper—20 ug vit. B_1	64·0	24·4	144·8	236·8	
		Lower—glucose soln. only	78·0	44·0	109·7	197·0	
10.	900	Upper—sugar soln. only	63·0	not estimated	61·0	132·7	
		Lower—50 ug vit. B_1	70·0		57·0	161·1	

* This is obtained by deducting from 324 mg (*i.e.* the amount of sugar in glucose, present in 6 c.c. of 5·4% glucose solution introduced into intestinal loops) the amount of glucose present in 1 c.c. of liquid taken but from the intestinal loops after 20 minutes and the amount present in the unabsorbed portion of the liquid left in intestinal loops after an hour.

showing a relatively greater amount of absorption of glucose in these experiments in loops containing vit. B_1. It is difficult to say without performing a different series of experiments why there is a greater absorption of glucose from some loops. This might be due to a greater amount of phosphatase in the walls of these loops, accelerating the speed of phosphorytation of glucose and accordingly the speed of its absorption.

It has been assumed in connection with experiments in Table I, that if vit B_1 be present in a loop beyond a certain concentration (which is near about 10 ug), it may combine with phosphate present therein and may thus lower the concentration of free phosphate in that loop. This assumption does not seem to hold good in the present series of experiments excepting in (7) and (9) in which the phosphate content after 20 minutes is lower in loops with 15 ug and 20 ug vit. B_1 respectively than in loops without it. The lower phosphate content in these loops is apparently due to a different reason, as mentioned above. This apparent anomaly between the experimental results of Tables I and II can be explained by the following considerations. It will be shown presently that glucose introduced into an intestinal loop causes an inflow of phosphates into the loop, much greater than what occurs after the introduction of phosphate-free Tyrode solution. If vit. B_1 be present along with glucose, the phosphate inflow is further accelerated, insomuch that even after combination of phosphate with vit. B_1 an excess of free phosphate is still left behind. But if glucose-phosphate combination and its consequential absorption is accelerated by certain unknown factors such as increased phosphatase content or peristaltic movement etc.), there will be a lowering of phosphate content and sugar content (as in experiments 4, 7 and 9 after 20 minutes). Again, if glucose phosphate combination be impeded (as, for example, when the phosphatase content is smaller than usual, there will be an excess of free phosphate and excess of glucose in the loop with glucose and vit. B_1 (as in experiments 6 and 8 after 20 minutes).

(ii) That the glucose content in 1 cc of the fluid collected 20 minutes after the introduction of experimental fluids into loops is less in the loop containing vit. B_1 in all the experiments excepting (6) and (8). This shows that vit. B_1 definitely helps the absorption of glucose, probably by stimulating the inflow of phosphates as shown before, and then helping its combination with glucose and consequential absorption. The anomalous cases of (6) and (8) have been explained before.

The results of estimation of phosphate and glucose in the residual fluid in the loops collected 1 hr. after its introduction show:—

(a) That in experiments (1), (2), (3), (4), (7) and (9) the phosphate content in loops with vit. B_1 is greater than in those without it, but in four other experiments the reverse is true. In two of the latter, viz., in experiments (8) and (10) the difference in the amount of sugar absorbed between the two loops in each case is so great that the lower value of the phosphate content in the loop with vit. B_1 may be explained as due to a much greater amount of sugar absorbed therefrom. But that this is not the whole explanation is evident from the fact that in experiments (2) (4) and (9), the difference in sugar absorbed from two loops in each case is almost as great as that in experiments (10) and yet the

phosphate content in loops with vit. B_1 is greater than in those without it. Apparently the phosphate inflow in these loops must have been greater than usual.

No explanation can be given for the anomalous behaviour of experiments (5) and (6) in which there is an excess of phosphate in loops without vit. B_1 and a greater absorption of sugar from these loops. It seems there is another variable factor which also controls sugar absorption from and phosphate inflow into the intestinal loops. The anomalous cases observed in these experiments are probably, due to a variation from the usual amount of this factor in the blood of certain animals. This factor is probably an adrenal cortical hormone.

(b) That in all these experiments excepting (5) and (6) the amount of sugar absorbed is always greater from the loop containing vit. B_1 than from the one without it. Again in all these experiments excepting (8) and (10), the amount of sugar absorbed is always greater from the loop containing a greater amount of phosphate, although the amount of sugar absorbed is not proportional to the amount of phosphate left behind.

(c) The amount of sugar absorbed after one hour from the intestinal loop of different animals shows great variation like the inflow of phosphates into these loops, the minimum amount of sugar absorbed being 99.3 mg. and the maximum amount 250 mg. Such a wide variation in the absorption of sugar from the intestinal loop was also observed previously by Davidson & Garry (1940) who noticed a variation in sugar absorption from 108 mg. to 306 mg. It is to be noted that the amount of sugar absorbed from the loops bears no relationship to the weight of the animal.

It is thus evident that it is difficult to decide from these experiments whether vit. B_1 or phosphate content in a loop is a more important factor in helping absorption of sugar from the intestinal loops. Both seem to influence the absorption of sugar.

Table III gives the average amount of phosphate or glucose in intestinal loops at varying intervals after the introduction of phosphate and sugar-free Tyrode solution or 5.4% glucose solution. The smaller average amount of phosphate in loops with vit. B_1 20 minutes after the introduction of modified Tyrode solution, might be due, as suggested before, to either of the following causes:

(a) As care was not taken in the experiments recorded in Table I to collect the fluid from the same portion of loops, *viz.*, either from the middle portion or from the sides, there would be necessarily a difference in the phosphate content in the fluid collected after 20 minutes from different portions on account of a slow rate of diffusion of phosphate from blood to loops, which prevents (1) an equilibrium (2) in phosphate concentration between different portions of fluid (3) from being attained. (b) As vitamin B_1 may easily combine with phosphate to form thiamin pyrophosphate, this combination would necessarily reduce the amount of free phosphate in the fluid and this reduction would be marked when the phosphate content in the loop is low, as after 20 minutes.

If the average amount of phosphate after 20 minutes in loops containing phosphate-free Tyrode solution with or without vit. B_1 be compared with that

TABLE III

Table III (obtained by calculation from tables I & II) (showing the *average* content of phosphate or glucose in intestinal loops containing 6 cc. of sugar and phosphate-free Tyrode solution with or without Vitamin B_1 or 6 cc. of 5·4 % glucose solution with or without vitamin B_1).

Amount of phosphate in ug in 1c.c. of Tyrode soln. collected 20 mins. after introduction.		Amount of phosphate in ug in the whole of remaining Tyrode soln. in loops after one hour.		Amount of phosphate in ug in 1 c.c. of glucose soln. collected 20 mins. after introduction.	
In loops with Vit. B_1	In loops without Vit. B_1	In loops with Vit. B_1	In loops without Vit. B_1	In loops with Vit. B_1	In loops without Vit. B_1
49·4	54·88	179·7	169·2	108·47	104·72

Amount of phosphate in ug in the whole of the remaining glucose soln. in loops after one hour.		Amount of Glucose in mg in 1 c.c. of glucose soln. collected 20 mins. after introduction.		Amount of Glucose in mg absorbed from the loops after one hour.	
In loops with Vit. B_1	In loops without Vit. B_1	In loops with Vit. B_1	In loops without Vit. B_1	From loops with Vit. B_1	From loops without Vit. B_1
194·85	170·44	43·92	49·07	192·6	162·4

in loops containing glucose solution with or without Vit. B_1, it will be observed, that the difference is very great, showing that glucose is a very potent factor causing the inflow of phosphates from blood into intestine. The difference in phosphate content after one hour between loops containing sugar and phosphate-free Tyrode solution with or without vit. B_1 and loops containing glucose with or without vit B_1 is not quite appreciable. This is due to the fact that after one hour a large amount of glucose is absorbed carrying with it phosphate in combination and thus reducing the total amount of phosphate present in the loops.

The average amount of glucose after 20 minutes in loops with vit. B_1 is less than that in those without it. This shows that more glucose is absorbed from the former than from the latter. This is confirmed by the fact that an appreciably large amount of glucose has been absorbed after an hour from the loop with vit. B_1 than from a loop without it.

Conclusion:

It is evident from Table III that if the average amount of phosphate inflow or absorption of glucose from loops with or without vit. B_1 be taken into consideration, then the irresistible conclusion is that vit. B_1 causes an acceleration of the inflow of phosphates and of absorption of glucose, but whether the greater absorption of sugar is dependent upon greater inflow of phosphates or independent of it, cannot be decided by the present series of experiments, as mentioned before.

In analysing the results of individual experiments recorded in Tables I and II, it has been shown that the majority of the experimental results are in support of the conclusion deduced above. Scrutiny and discussion of the anomalous behaviour presented by certain experiments also lead to the same conclusion in most of these latter cases. There are few cases which could not be explained excepting by the supposition that there are other variable factors which control the inflow of phosphates into and absorption of glucose from the alimentary canal.

SUMMARY

1. The influence of thiamin on phosphate inflow into the intestinal canal and the absorption of glucose therefrom has been studied on rabbits in two consecutive loops of jejunum after they were washed with warm Tyrode solution without phosphate and glucose.

2. In one series of experiments 6 cc of Phosphate and glucose-free Tyrode solution with or without vitamin B_1 was introduced in two consecutive loops, and the phosphate content of these loops was ascertained after 20 minutes and also after an hour. (Vide Table I).

The analysis of results recorded in Table I show that the phosphate contents after 20 minutes in intestinal loops with or without vitamin B_1 or with different amounts of vitamin B_1 are inconstant and irregular and after an hour the amount of phosphate in loops with 10 ug vit. B_1 is greater than that in loops without the vitamin, but in loops containing 15 ug or more of vit. B_1 the amount of phosphate is less than in those without it and between loops containing dif-

ferent amounts of vit. B_1 that loop which contains a greater amount of vit. B_1, has less amount of phosphate.

It has been suggested that the inconstant results about the phosphate content in loops after 20 minutes are probably due to the collection of liquid from different portions of these loops. The varying results regarding the effect of vit. B_1 on the inflow of phosphates have been explained by the assumption that vit. B_1 promotes the inflow of phosphates, but if the amount of vit. B_1 be in excess of a certain concentration, it may combine with phosphate and thus reduce the concentration, of free phosphate in the fluid.

3. In another series of experiments 6 cc of 5.4% glucose solution (hypertonic) with or without vitamin B_1 was introduced in two consecutive loops and the phosphate and glucose contents of these loops were ascertained after 20 minutes and also after an hour, as before (Vide Table II).

The analysis of results recorded in this table reveals the following:

(i) Fluid (1 cc) collected after 20 minutes from loops containing vit. B_1 shows a greater amount of phosphate corresponding to a smaller amount of sugar in 5 experiments viz, (1), (2), (3), (5) and (7), a less amount of phosphate corresponding to a definitely smaller amount of sugar (in comparison with that of the consecutive loops) in 2 experiments, viz., (4) and (9) and a greater amount of phosphate corresponding to a greater amount of sugar in 2 experiments, viz., (6) and (8).

(ii) Residual fluid collected after 1 hour from loops containing vit. B_1 shows a greater amount of phosphate and a greater absorption of sugar in 6 experiments, viz., (1), (2), (3), (4), (7) and (9), a less amount of phosphate and a greater absorption of sugar in 2 experiments, viz., (8) and (10) and a less amount of phosphate corresponding to a less absorption of sugar in 2 experiments, viz., (5) and (6).

While these results point to the conclusion that vit. B_1 has some relationship with the inflow of phosphates into and the absorption of sugar from the intestine, yet the anomalous behaviour noticed in certain experiments would lead one to think that there are other factors which are concerned in both phosphate inflow or sugar absorption. These factors are probably phosphatase content of the intestinal wall and des-oxy-corticosterone liberated from the adrenal cortex. The posphate inflow into intestinal loop and absorption of sugar therefrom after one hour show wide variations and bear no relationship to the weight of the animal.

Table III has been obtained by calculation from Table I and II and gives the average figures re phosphate inflow and sugar absorption. These average figures are predominantly in favour of the influence of vit. B_1 on phosphate inflow and sugar absorption.

Acknowledgements

We are indebted to the Indian Research Fund Association, Public Health Department and the Bengal Government (Medical Department) for grants for defraying the expenses of this research. We are also grateful to Mr. D. P. Sadhu M.Sc for helping us in the dissection of the animal and in the carrying out of the estimations.

The Indian Journal of
Physiology and Allied Sciences
Vol. I. No. 1, 1947.

OBSERVATIONS ON THE ASSAY OF PROLACTIN BY THE CROPGLAND STIMULATION IN INDIAN PIGEONS.

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Introduction

Manufacture on a small scale of glandular products of the type of adrenaline, posterior pituitary extract and thyroid extract, has been successfully done in India and their bioassay is being systematically carried out in this laboratory. Of late, attempts are being made to isolate various active fractions from the anterior pituitary bodies. In order to help research chemists in the isolation of some of these specific hormones in pure state, it has been necessary to introduce in this laboratory certain newer methods of bioassay required in this connection.

Prolactin, galactin or mammotrophin is a distinct hormone elaborated by the anterior lobe of the pituitary gland and it has been clearly established that this hormone is primarily concerned in the regulation of milk secretion of animals. Folley (1938) has adduced evidence to show that both the yield and the fat-content of milk are increased by administering prolactin to lactating cattle. The importance of this observation to the dairy industry is obvious and reports from reliable sources indicate that the use of prolactin is being encouraged in Soviet Russia and in certain European countries to increase milk-output in cattle. If prolactin could be isolated in sufficiently large quantities, experiments on similar lines could also be started in India with beneficial effects on Indian dairy industry.

As a preliminary step to the determination of the potency of these preparations, the establishment of the 'crop-gland stimulation' technique on Indian pigeons with International standard prolactin has been attempted for the first time by us. The present paper embodies our observations in this regard and presents a dose-response curve for the standard which may be conveniently adopted for reference purposes by other investigators working under Indian conditions.

Experimental

Materials and Methods:—

(1) *Birds and their treatment*:—The experiments mentioned here were done during the months of July and August. Both male and female pigeons, 4 to 6 months old, weighing between 150 g. to 300 g were kept on mixed diet of grains before and during the period of experiment. As the birds were procured

from outside sources, their exact litter was not known. The majority of them however belonged to mixed breeds. After the birds had maintained a constant level of weights for about a week, 2 injections daily of varying doses of prolactin for a period of 4 days were given into the pectoral muscles at 10 A.M. and 4 P.M., alternating the side of injection every day. On the 5th day at about 10 A.M. (18 hrs. after the last injection), the birds were sacrificed and the crop glands dissected out and weighed.

(2) *Solutions employed*:—The International Standard Prolactin tablets* (1939) were weighed accurately and dissolved in sterile redistilled water with a drop or two of normal sodium hydroxide solution. The final solution was adjusted to a concentration of 1 in 1000 (10 Units/c.c.) with a pH between 8.4 and 8.5 This was then stored at a temperature between 0°C and+5°C, during the period of investigation with each series of tests.

(3) *Dissection of the Gland*:—The dissection of the crop-glands was conveniently done by removing the feathers from the region of the throat and then exposing the whole crop-sac by a mid-line vertical incision down to the sternal notch. The sac which lay almost immediately beneath the skin was then carefully dissected out *en bloc* with a blunt dissector and liberated from the fascial attachments and fatty tissue on its dorsal surface. It was then incised from the upper and lower gullets, opened ventrally in the direction of the two openings, and freed from food grains by a gentle spray of water.

The glands are situated in an oval area, about 2 cm. in diameter on each side of the posterior wall of the sac. It is not always easy to locate the exact glandular area, as, normally, it consists of mucous folds of slightly greater thickness than the neighbouring areas. It is desirable therefore to compare the normal glands of the control pigeons with the proliferated glands of the prolactin-treated birds when the correct definition of the outline of the gland becomes comparatively easy. After stimulation, the gland becomes greatly hypertrophied, and bulges out like a miniature cauliflower, often containing a thick layer of creamy exudate covering a highly vascular surface. The crop glands are then carefully removed as a whole, soaked between two folds of filter paper and weighed quickly in weighing bottles. The alternative suggestion of Emmens (1939) for fixing the glands in Bouin's fluid prior to the weighing was not observed.

Results:—

Eighty pigeons were used for the whole series of experiments, of which, *sixty-seven* birds have been used for the construction of the dose-response curve. The weight of the crop-glands of pigeons in their normal condition and after treatment with different series of dosages of prolactin are given in the Table. The histological appearances of normal and proliferated glands are shown in the accompanying Plate. A dose-response curve, with the mean percentage increase in the weight of the crop glands as ordinates and the dosage of prolactin in units as abscissae, is also given.

* Obtained from the National Institute of Medical Research, Hampsteed, London one tablet =100 units. 1 unit=specific activity contained in 0·1 milligramme (=100 gamma) of the standard preparation.

TABLE

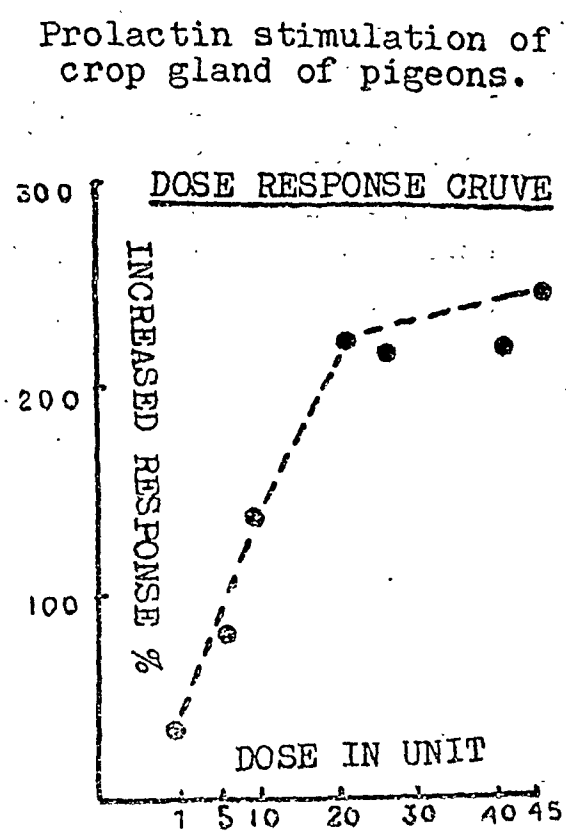
Effect of intramuscular Prolactin* injections on the crop gland stimulation of pigeons.
(2 injections per day for a period of 4 days)

Total dose Unit|100g. wt. Number of pigeons	nil		0·4		2·0		4·0		8·0		12·0		16·0		20·0	
	Weight of crop glands (mg.)	Total dose per bird (Units)	Weight of crop glands (mg.)	Total dose per bird (Units)	Weight of crop glands (mg.)	Total dose per bird (Units)	Weight of crop glands (mg.)	Total dose per bird (Units)	Weight of crop glands (mg.)	Total dose per bird (Units)	Weight of crop glands (mg.)	Total dose per bird (Units)	Weight of crop glands (mg.)	Total dose per bird (Units)	Weight of crop glands (mg.)
1.	902	1·2	1426	5·2	1784	9·4	2622	20·0	4519	24·0	2004	42·4	3250	50·6	2634
2.	1039	1·2	1371	4·9	1684	9·4	2808	19·2	3228	25·8	2413	40·0	3473	44·0	2654
3.	895	0·8	1272	5·6	2213	8·8	1699	22·0	4068	22·8	2874	40·0	2823	36·0	2076
4.	997	1·0	1537	4·0	1660	9·2	2049	16·8	3097	21·6	2028	20·8	2704	44·0	2339
5.	966	0·9	1097	5·3	1817	9·6	1929	21·6	3042	34·8	4335	38·4	2350	36·0	2701
6.	774	0·8	1269	5·5	1572	9·8	2471	19·6	4199	29·4	3259	45·6	3726	40·0	2675
7.	859	1·1	1090	3·6	1432	9·8	1769	17·6	2078	23·4	2385	30·4	3104	56·0	5848
8.	994	1·0	1092	4·3	1571	11·6	2249	22·2	2557						
9		1·1	1118			11·0	2420	21·4	2497						
10.		1·1	1150			9·0	2646	22·8	3055						
Average:—	922	1·0	1240	4·8	1716	9·7	2260	20·3	3234	26·0	2756	36·8	3061	43·8	2989
	* (396|100g)														
Mean increase in crop weight: mg|100g weight		136	51§	331	68	557	138	925	243	850	207	930	167	944	373
Percentage increase.		31·5	13	83	17	139	35	231	61	213	52	233	42	236	93
Percentage Error.		41		20		25		26		24		18		39	

Note:—The degree of accuracy obtainable in the assay of prolactin by this method can be judged from the last horizontal column. The optimum dose recommended in carrying out routine assay of unknown samples is 4 Units\|100g which gives an increase in crop weight of 139 per cent. with an error of 25 per cent.

* From this fig. it appears that the average weight of birds is taken to 233 g. nearly.

§ If the average weight of birds be taken 233 g., then the mean increase works out to 136 plus minus 51.



It is evident that progressively greater responses are obtained at the lower level of dosage than at the higher. Increase in response is greater from the first 3 series of dosage in proportion to the amount given and the dose-response curve is steep and linear in this region. With further increase in the level of doses, as in the last three series, the response is less in proportion to the amount given and the graph is almost horizontal. It is interesting to note that Dyer (1936) and Riddle *et al* (loc. cit) also observed a tendency for regression of prolactin effect when the injections were continued beyond a certain number of days and the optimum dose-level exceeded. In the present investigation with the International Standard Prolactin solution, a maximum response of about 231 per cent. increase over the normal crop weight was obtained with a dosage of 8 Units/100 g. weight of the pigeon. It would thus appear that the selection of dosages for the assay of unknown samples of prolactin should be made at a lower level than this where submaximal effect is produced. A dosage of 4 units/100 g. weight of the bird (given in 8 injections during 4 days), still gives a 139 per cent. increase over the normal crop-weight of pigeons. This may, therefore be considered to be a satisfactory dose-level for the assay of prolactin. No discrimination of sex in the choice of birds is considered necessary by the authors, as both male and female birds are equally sensitive to prolaction stimulation. The question of distribution of doses and duration of injections is, however, important. Of the various ways of preparing the birds for optimum crop-gland stimulation—one injection daily for 7 days, one injection per day and on alter-

PLATE

Morphology of normal and proliferated crop-gland of pigeons.

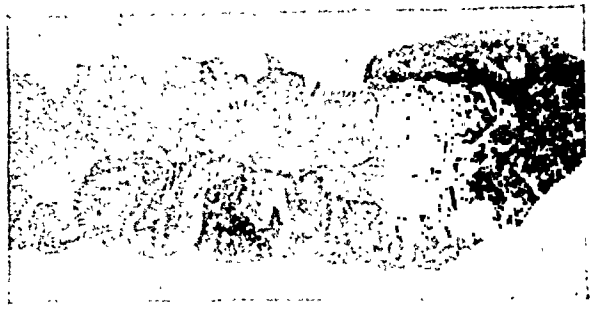


Fig. 1. Normal glands:—A growing layer of squamous epithelium covering a muscular layer. The epithelium is arranged in papillae. On the outer layer of these a few keratinised cells and in the loose tissue of the central crore some blood vessels are to be seen. No acini or duct like structure is present any where.

Fig. 2. Gland stimulated by a median dose of prolactin (4 units/100g. weight of pigeon):— Moderate hypertrophy and hyperplasia of the epithelium with irregularity in shape and size of papillae and formation of papillomatous growths. Invasion of the muscular layer by isolated patches of squamous epithelium.

Fig. 3. Gland stimulated by a maximal dose of prolactin (8 units/100g. weight of pigeon):— Very marked hypertrophy and hyperplasia of the papillae, many areas resembling papilloma. Flattening of the muscular layer by epithelial over-growth. Complete absence of any glandular structure in the picture.

nate days for 4 days,—it was observed that for equivalent unitage the method of two injections per day, for a period of 4 days (followed in the present study) is the most suitable.

Summary

1. A dose-response curve showing the relation between various doses of the International Standard of 'prolactin' and the percentage increase in the weight of the crop-glands of pigeons has been drawn as a preliminary to the future assay of lactogenic hormones in India.

2. Details of the technique, the range of dosages of prolactin and the precautions to be taken in the dissection of the crop glands to obtain uniform results are also given.

3. It has been found that a total dose of 4 units of the International Standard per 100 g. weight of pigeons, given intramuscularly in 8 injections during 4 days is satisfactory dose-level for the assay of prolactin samples. In this range the response is linear and hence sharp distinction between two graded doses is practicable. From the figure of standard error for the above dosage, it appears that prolactin could be assayed biologically on 10 pigeons with a permissible limit of plus minus 25 per cent.

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ASCORBIC ACID CONTENT OF GARDEN ROSE HIPS.

By

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Wang Chen Fa (1944) reported very high concentration of Vitamin C in Chinese Rose Hips, collected from the sides of fields and roads and the slopes of the hills in the region of Koloshan, Chungking. The Ascorbic acid content of the Chinese Rose Hips averaged to 19·93 mgms, per gram of air dried Rose Hips of the green varieties. Following this finding a few varieties of Garden Rose Hips were examined for their Vitamin C content by us. It was found that the ascorbic acid content of the Garden Rose Hips is negligibly small in comparison with that of Chinese Rose Hips. It was also found by us that the Vitamin content of the garden rose hips varies from plant to plant, and depends to a great extent on their maturity.

EXPERIMENTAL PROCEDURE:—

After removing the dried clayx, the fresh hips were weighed and ground in a motar with washed sea sand and 3-5 C.C. of 10% Trichloro acetic acid. The paste was then taken in a centrifuge tube with more Tricholoro acetic acid and centrifuged. supernatant liquid was then decanted into a measuring flash, the residue was taken up with more Trichloro acetic acid well mixed and again centrifuged. The washings were then added to the original solution in the measuring flask and made up to a definite volume. The Ascorbic acid content was then determined by titrating against a standard Indo phenol dye.

Results:—

Variety	mgms of ascorbic acid/100gms. of the green hips.
Sombrarel, I	36
Betty uprichard H.T.	17
Red pet Poly	18
Do (Young)	25
Doranga C.	72
Else Poul sen (Poly)	41
Dutches of Willington H.T.	39
Golden Fairy Poly. (Young)	46
Do (Old)	23
Picture H.T.	105
Madam Edward Harriot-A.B.	18
Grassan Teplitz H.T. (Young)	62
Do (Old)	35
Gorgeons H.T.	60

Conclusion:—

The results show a meagre concentration of Ascorbic acid in the Garden Rose Hips.

Reference:—

Wang Chen Fa., Science and Culture (1944) No. 8, Vol. 9.

The Indian Journal of
Physiology and Allied Sciences
Vol. I. No. 1, 1947

INFLUENCE OF VITAMIN B_1 ON DETOXICATION BY GLUCURONIC ACID PRODUCED IN LIVER.

By

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Liver being the largest organ in the body is naturally a storehouse of many substances which are required in its metabolic processes. Thus it is a depot for glycogen, vitamin A, vitamin B_1, riboflavin etc. Liver is also the seat of many metabolic reactions in the body, some of which are influenced by vitamin B_1, such as fat metabolism (Mc. Collum, 1939). An important function of liver is detoxication of many harmful substances by conjugating them with glucuronic acid. Glucuronic acid is apparently formed in liver from d-glucose by oxidation of its terminal-CH_2OH group into COOH group. What factors are concerned in the control of the formation of this acid for detoxication purposes is not known, but it has been found that a dose of CCl_4 which is fatal if the liver is fatty, is without effect if the liver is filled with glycogen (Wright 1940). It may be inferred from this that when there is a rich store of glycogen, a large amount of glucuronic acid may be formed for detoxication. It has been conjectured that amongst the various factors which may control the formation of this acid from glycogen vitamin B_1 and riboflavin which are stored in large amounts in liver may have some role in this process and therefore in the detoxication function of liver. In the present paper the possibility of vitamin B_1 in helping this function of liver has been investigated.

Experimental

As liver is the seat of formation of glucuronic acid (Hemingway et al, 1934) and as it is considered that vitamin B_1 or riboflavin or both may be necessary for the formation of this acid from glycogen, it may then be expected that there would be a difference in glucuronic acid excretion between B_1-avitaminosed animals and normal animals, particularly after the intake of a substance like chloral hydrate which calls up the production of glucuronic acid for its detoxication. But as the amount of glucuronic acid required for the detoxication of the little dose of chloral hydrate given to these animals is quite small, it is likely that the production of such a small amount of glucuronic acid will persist even in B_1-avitaminosed animals, so that the difference in excretion of this acid between two sets of animals would be inappreciable. If, however, a large portion of the liver tissue be damaged, as by the intake of CCl_4, then the production of glucuronic acid is expected to be so very restricted, particularly in animals not receiving vitamin B_1, that there may be appreciable difference in the excretion of glucuronic acid between the two sets of animals. Experiments were accordingly devised to put to test these considerations.

Two groups of young rats of nearly the same weight (one group containing 6 and the other 10 rats*) were put on vitamin B_1-deficient diet. *viz.* casein 15%, dextrin 65.5%, auto-claved yeast—10.0%, salt mixture—2.5%, ghee—5.0% and cod-liver oil—2.0% and a few drops of lemon juice. Each member of the first group (containing 6 rats) received daily a supplement of 10 ug vit. B_1 (to be called the normal group), the other group not having received any such supplement (hence, the deficient group). The members of each group were arranged into pairs,§ each pair being kept in a metabolism cage, and the urine of each pair was estimated periodically for glucuronic acid for eight weeks in the course of which all the animals of the deficient group showed definite symptoms of vitamin B_1 deficiency (two of them having succumbed at the end of the 7th week).

Two of the animals showing definite symptoms of vitamin B_1 deficiency were each given per mouth 1.5 cc of CCl_4 for damaging their liver, but unfortunately both the animals died as a result of the intake of this dose of CCl_4. The dose of CCl_4 was, therefore, considerably reduced and after a few experiments with animals a dose of 0.176 cc per rat was selected. This dose was given for a day only to all the rats of both the groups, so that their liver might be damaged. The next day, all of them were each given per mouth a single dose of chloral hydrate (200 mg per Kg of body-weight) and the urine voided by the animals during the subsequent 24 hours and the following day was estimated separately for glucuronic acid. The difference in glucuronic acid excretion was not found to be appreciable. It was considered that CCl_4 might take a little

* A larger number of rats were put on the deficient group as it was apprehended that some of them might succumb after they became deficient.

§ Instead of puting one animal in each cage, a pair were kept for the following reason:— The amount of urine void by one animal per day is so small that it was apprehended that when the floor of the cage, the sides of the funnel below the cage and the beaker in which the urine was collected would be washed down with the smallest possible volume of water and collected in a measuring cylinder or flask, the amount of glucuronic acid in its aliquot part would be too small to be estimated.

longer time than a day or two for its damaging action on liver to be completed, so that unless sufficient interval is allowed between the administration of CCl_4 and chloral hydrate, glucuronic acid excretion may not be considerably affected. Accordingly, an interval of 5 days was allowed between the administration of another dose of CCl_4 and chloral hydrate to all the animals, and the urine of the subsequent 24 hours and the following day was estimated as before, for glucuronic acid. Even now the difference in excretion of this acid between the two groups of animals was not appreciable. It was accordingly believed that a single dose of CCl_4 might not damage sufficiently the liver of the animals so as to bring out the expected difference in excretion of glucuronic acid between these two groups of animals. Thus CCl_4 (in a dose of 0.176 cc per rat) was given daily to all the animals for a week after which chloral hydrate was also administered in the same dose as before for a week, the urine of these animals being examined for glucuronic acid throughout the whole period of administration of chloral hydrate and also for 24 hours following the cessation of its administration. The difference in glucuronic acid excretion between these two groups of animals was now found to be quite appreciable.

Estimation of Conjugated Glucuronic Acid

Chloral hydrate, $CCl_3\,CH(OH)_2$ or chloral, $CCl_3\,CHO$, is detoxicated in the body as follows:

by reduction and then reaction with glucuronic acid

$$\begin{matrix} CCl_3\,CH(OH)_2 \\ \text{or} \\ CCl_3\,CHO \end{matrix} \qquad CCl_3\,CH_2OH + CHO.(CHOH)_4.COOH$$

Tri-chlor-ethyl alcohol — glucuronic acid

$$CCl_3CO(CHOH)_4.COOH$$

urochloralic acid

This conjugated glucuronic acid is estimated according to Salt's modification of Tollen's naphthoresorcinol test (Salt, 1935) with such modifications as were found necessary and the method is given below:—

The urine, collected as mentioned before in a measuring cylinder, is rendered slightly acidic with acetic acid and its volume noted. A measured amount is pipetted into each of four 10 cc centrifuge tubes to which are added respectively 0·25 cc, 0·5 cc, 0·75 cc and 1·0 cc of 5% lead-acetate. The contents are then mixed well and centrifuged. After the precipitate has settled down, a drop of lead acetate is added to each of these tubes. If any of them does not show any precipitation at this stage, then the tube may be taken to have undergone complete precipitation by the previous addition of lead acetate. But if there be precipitation in all these test tubes, then another cc of 5% lead-acetate is added to each of these tubes. All these tubes are then tested as be-

fore by the addition of one drop of the lead acetate solution for finding out in which of them precipitation is complete. In this way, the addition of an appreciable excess of lead acetate is prevented. The supernatant fluid from the tube in which precipitation is found to be complete, is transferred to another centrifuge tube and NaOH solution (approximately normal) is then added drop by drop until permanent precipitation is noticed. Then 3 cc of 10% basic lead acetate is added and mixed thoroughly in the tube which is then centrifuged. To ensure complete precipitation, basic lead acetate solution is added slowly, drop by drop, till precipitation no longer takes place and the tube is once more centrifuged. The supernatant fluid is decanted off, the precipitate washed in the tube by shaking thoroughly with 5 cc water and again centrifuged. The precipitate is then transferred to a long test tube by shaking twice with 5 cc of 50%HCl each time. 0·5 cc of 1% naphtho-resorcinol solution is then added and mixed thoroughly. The tube is then heated in boiling water for 5 minutes, then cooled in running water; 10 cc of ether is next added to it with shaking and is allowed to separate. The separated ethereal layer is then removed to a dry test tube and examined immediately with Pulfrich's photometer, for otherwise the bluish violet color that is formed fades away on standing. The color may be stabilised, when necessary, by the addition of a small amount of hydroquinone to the solution.

The blue-violet color of the solution is estimated by Pulfrich Photometer, using filter S—53 which filters off light of wave-length 530 uu, and the result is expressed in terms of color absorption (i.e., the extinction coefficient) which, according to Salt is nearly proportional to the quantity of glucuronic acid present in the solution. A better approximation would have been obtained if definite amounts of glucuronic acid were treated with naphtho-resorcinol solution and the color developed matched with that obtained from the unknown solution. As glucuronic acid was not available, the indirect method, mentioned above, has been followed.

RESULTS

Tables I, II, III and IV give the results of these four series of experiments relating to glucuronic acid excretion (1) in normal and vitamin B_1-deficient animals, (2) in both these groups of animals after the intake of a single dose of CCl_4 (0·176 cc per rat) by each rat followed on the next day by an intake of a single dose of chloral hydrate (200 mg per Kg of body-weight), (3) in both these sets of animals after the intake as before of a single dose of CCl_4 followed after 5 days by an intake of a single dose of chloral hydrate and (4) in both these sets of animals after each of them received for a week a dose of CCl_4 (0·176 cc per rat) per day and then for another week a dose of chloral hydrate (200 mg per Kg) per day.

TABLE I

(Giving the average extinction coefficients of the blue-violet colored solutions obtained from urines of normal and vitamin B_1-deficient animals during

a period of 8 weeks, these coefficients being proportional to the amount of glucuronic acid present in these urines)*

Average daily excretion of glucuronic acid in terms of the extinction coefficients.

Normal animals (Basal diet+10 ug vit. B_1 daily) divided into 3 pairs, A, B, and C.

	Minimum	Maximum	Last week's average	Average for 8 weeks.
A	0·8	3·4	2·17	2·2
B	1·3	2·8	1·55	2·2
C	0·9	2·9	2·1	1·91

Deficient Animals.

(Basal diet without vit. B_1)

Divided into 5 pairs, D, E, F, G and H.

	Minimum	Maximum	Last week's average	Average for 8 weeks.
D	0·97	2·7	2·5	1·66
E	0·81	2·5	1·6	1·62
F	0·95	3·0	1·7	1·70
G	0·60	2·4	0·62	1·61
H	0·58	3·2	0·58 (average of the 7th week)	2·05 (average for 7 weeks) (The animals, of the pair, H died at the end of 7 weeks.

TABLE II

(Showing the average daily excretion of glucuronic acid after the intake of a single dose of CCl_4 followed after 24 hours by the intake of a single dose of chloral hydrate)

Average per day of two days' excretion†:—

Normal Normal group Normal		Deficient Deficient group Deficient	
A	1·5	D	1·6
A	1·5	D	1·6
B	1·46	E	1·52
C	1·52	F	1·48

TABLE III

(Showing the average daily excretion of glucuronic acid after the intake of CCl_4 followed after 5 days by the intake of a single dose of chloral hydrate)

Average per day of two days' excretions:—

	Normal group		Deficient group
A	1·8	D	1·62
B	1·5	E	1·55
C	1·6	F	1·52

* As the excretion of glucuronic acid fluctuated from day to day, only the minimum and the maximum excretion of each pair of rats during a 24-hour period in the course of the 8 weeks during which all the animals of the deficient group showed symptoms of vitamin B_1 deficiency, are given. and also the average daily excretion during the last week and the average daily excretion of these 8 weeks are recorded.

† The pair G which became highly deficient were given each a dose of 1·5 cc of CCl4, This resulted in their death. Accordingly a much reduced dose of 0·176 cc of CCl_4 was next given to all these animals.

TABLE IV

(Showing the average daily excretion of glucuronic acid after the daily intake of CCl_4 for a little more than a week followed by the daily intake of chloral hydrate for a week)

Average per day of excretions for 8 days:—

Normal group		Deficient group	
A	2·2	D	1·4
B	1·8	E	1·25
C	2·0	F	1·2

Discussion

In Table I the excretion of glucuronic acid in animals before the administration of chloral hydrate is given. It is curious that the average excretion of this acid is higher in the case of both normal and deficient animals than when these animals were treated with CCl_4 and chloral hydrate (a single dose of each), although it is expected that the excretion would rise as chloral hydrate stimulates its production. This can be explained by the supposition that even a single dose of CCl_4 has impaired to some extent the efficiency of liver in forming glucuronic acid. In Table IV the excretion of glucuronic acid by normal animals after they had received nine administrations of CCl_4 (seven of which were given on successive days) is almost as high as in Table I. This is because the intake of chloral hydrate on seven successive days has strained the liver tissue to the utmost to form glucuronic acid for its detoxication. In Table I it is seen that the excretion of glucuronic acid by normal animals is slightly but definitely greater than that by deficient animals. This apparently shows that vitamin B_1 has a role in its production. But in Tables II and III no definite difference in excretion between these two groups of animals can be made out. This is probably due to the damaging effect of CCl_4 being predominant, the effect of vitamin B_1 deficiency has been masked and also a single dose of chloral hydrate has failed to bring out the difference in glucuronic acid producing power of the livers of these 2 sets of animals. When the livers are intensely damaged by the intake of CCl_4 for a number of days in succession, the difference in their capacity of production of this acid is well brought out when they are whipped into activity by repeated doses of chloral hydrate (vide Table IV). The production by liver of glucuronic acid under the above conditions of experiment is comparable to the production of a substance or substances by two factories of which the working capacity of labourers is different, after partial or intensive damage of their machinery by, say, aerial bombardment. If the demand for the substance produced in these factories be small and if the damage of the machinery be slight, the difference in the working capacity of the labourers cannot be discovered, as the small demand can be satisfied easily. But when the damage is extensive and the demand high, the difference in the working capacity is easily discovered.

It is obvious from Table IV that inspite of a significant decline in output of glucuronic acid in the deficient group of animals after a long period of vitamin B_1 avitaminosis, the production of glucuronic acid by them is still quite sufficient although less than that of normal animals. This leads to the conclusion that there

are other potent factors besides vitamin B_1 which are concerned in its production. One such factor may be riboflavin which is also stored in large amount in liver. It has been noticed by the authors that glycogen storage in liver in vitamin B_1-deficient animals, is not impaired. Accordingly, the possibility of decline in glucuronic acid excretion in Table IV due to any shortage of glycogen in vitamin B_1-deficient animals, is ruled out.

Summary

(1) The glucuronic acid excretion of normal and vitamin B_1-deficient animals, was studied for 8 weeks. The excretion by the former is slightly though definitely higher than the latter.

(2) When the animals are given a dose of CCl_4 which is known to damage the liver and a dose of chloral hydrate which calls up the production of glucuronic acid for its detoxication, either on the following day or after an interval of a few days, it is found that their glucuronic acid excretion declines but the difference in excretion between these two groups of animals is no longer appreciable. An explanation is suggested for these effects of CCl_4 and chloral hydrate.

(3) When these animals are first given daily CCl_4 for a week or so and then chloral hydrate in the same way for also a week or so the difference in excretion of glucuronic acid by these two groups of animals becomes quite appreciable, that of the deficient animals being appreciably less than that of the normal animals.

Conclusion

It is quite obvious from these experiments that vitamin B_1 has a role in the production of glucuronic acid by liver. As in animals highly deficient in vitamin B_1 a sufficient amount of glucuronic acid is still excreted, it is concluded that there are other more potent factors concerned in the production of glucuronic acid by liver. It may be rib oflavin which is stored in large amount in liver.

Acknowledgments

We are indebted to I. R. F. A. for a grant for this enquiry. We are also grateful to Messrs. Volkart Brothers, sole agents of Hoffman La Roche, for the supply of vitamin B_1 tablets required in this enquiry.

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1938	Crew, Prof. F. A. E.	Institute of Animal Genetics, Edinburgh.	
1938	Cullis, Prof. Winifred	School of Medicine for Women, London.	
1944	Hill, Prof. A. V.	Secretary, Royal Society, London.	President, 1934—1940
1940	Mahalanobis, Prof. S. C.	90, Park Street, Calcutta.	Vice-President, 1937
1944	McGilchrist, Lt.-Col. A. C.	9, Mandevil Gardens, Calcutta.	
1938	Poulton, Dr. E. P.	Guy's Hospital, London.	
1938	Straub, Prof. W.	University of Munich, Germany.	

ORDINARY

1943	Acharyya, Dr. G.	Prince of Wales Medical College, Patna.	
1935	Prof. B. Ahmad	Council of Scientific & Industrial Research, University Buildings, Delhi.	Secretary, 1936, 1937 & 1939
1942	Prof. Alahasingari	Medical College, Vizagpattam, Madras.	
1937	Aykroyd, Dr.	Director, Nutrition Research Laboratory, Coonoor.	V. President, 1934, 1944
1938	Banerji, Dr. D. N.	Pathology Dept., Carmichael Medical College, Calcutta.	
1944	,, Dr. P. K.	Dept. of Physiology, Carmichael Medical College, Calcutta.	
1938	,, Dr. Sachchidananda	A. Mitra Diabetic Research Scholar, School of Tropical Medicine, Calcutta.	Treasurer, 1942, Asst. Secy., 1940
1934	,, Prof. S. M.	Cuttack Medical College, Orissa.	Asst. Secy., 1936, 1937, Secy. 1940-42, Treasurer, 1936, 1944, Vice-President, 1945—46
1942	,, Principal T. N.	P. O. W. Medical College, Patna.	
1944	Basu, Dr. B. N.	Dept. of Anatomy, Carmichael Medical College, Calcutta.	
1934	,, Prof. N. M.	Presidency College, Calcutta, Dept. of Physiology.	Secy., 1934—35, Vice-President, 1938—40, 1944—46, President, 1942—43
1934	Bera, G. N.	Presidency College, Calcutta, Dept. of Physiology.	Asst. Secy., 1944
1934			
1937	Bhatia, Lt.-Col. S. L.	Inspector-general of Civil Hospitals, Assam.	Vice-President, 1937, 1939, 1940, 1944—46, President 1946

Year of Election	Name	Address	
1944	Bhattacharjya, Dr. K. P.	P. O. W. Medical College, Patna.	Secy., 1934, Vice-President, 1935—1944—45
1934	„ Prof. N. C.	19, Hindusthan Road, Calcutta.	
1943	„ Kaviraj Rajendranath	110|2A|2, Amherst Street, Calcutta.	
1940	Biswas, Rishikesh	School of Tropical Medicine, Calcutta.	
1934	Bose, Prof. G. S.	University College of Science, Calcutta.	
1934	Brahmachari, Dr. N. K.	6, Taltala Avenue, Calcutta.	Asst. Secy., 1934, Secy., 1945—46
1934	„ Dr. P. N.	19, Loudon Street, Calcutta.	
1937	Burridge, Prof. W.	King George's Medical College, Lucknow.	Vice-President 1939, 1942, 1943
1944	Chakravarti, Dr. A. R.	1, Furiapukur Street, Calcutta.	
1944	„ Dr. M. L.	Dept. of Physiology, Campbell Medical School, Calcutta.	
1935	„ Prof. Murari	Medical College, Agra.	
1934	Chatterji, Dr. B. B.	82, South Road, Entally, Calcutta.	Treasurer, 1943, Secy., 1944 & 1945
1944	Chowdhury, Dr. N. K.	Dept. of Pharmacology, P. O. W. Medical College Patna.	Secretary, 1946.
1934	Das Dr. N. N.	University College of Science Calcutta.	Secy., 1942 & 1943, Vice-President, 1944
1934	De, Prof. P.	Calcutta Medical College, Calcutta.	
1939	„ U. N.	School of Tropical Medicine.	
1941	Dikshit, Dr. B. B.	Haffkine Institute, Bombay.	
1942	Ghosh, Prof. B. N.	Dept. of Pharmacology, Carmichael Medical College, Calcutta.	
1942	„ Prof. G. K.	Dept. of Anatomy, P. O. W. Medical College, Patna.	
1942	Ghosal, Dr. S. M.	P. O. W. Medical College, Patna.	
1942	Grewal, Dr. K. S.	Dept. of Pharmacology, King Edward Medical College, Lahore.	
1942	Guha, Dr. A. K.	P. O. W. Medical College, Patna.	
1934	„ Prof. B. C.	University College of Science, 92, Upper Circular Road, Calcutta.	
1942	Gupta, Captain A. K.	C|o. Prof. U. M. Gupta, P. O. W. Medical College, Patna.	
1938	„ Dr. J. C.	Carmichael Medical College Hospital, Calcutta.	
1942	„ Dr. T. C.	Dept. of Physiology, P. O. W. Medical College, Patna.	
1942	Hai, Khan Sahib Dr. Md. Abdul	Dept. of Pharmacology, P. O. W. Medical College, Patna.	Asst. Secy., 1946
1942	Mahalanobis, S. K.	90, Park Street, Calcutta.	
1940	Lahiri, Dr. K. D.	P. O. W. Medical College, Patna.	
1934	Maitra, Dr. J. N.	1, Corris Church Lane, Calcutta.	
1942	„ S. R.	Dept. of Physiology, University College of Science, Calcutta.	Treasurer 1946
1944	Mitra, Bholanath	Dept. of Physiology, Carmichael Medical College, Calcutta.	
1944	„ Prof. B. K.	Islamia College, Calcutta.	
1944	„ Prof. Subodh	3, Chowringhee Terrace, Calcutta.	
1942	„ Dr. Kalidas	Nutrition Officer, Patna.	
1934	Mukherjee, Dr. B.	Biological Standardisation Laboratory, All India Institute of Hygiene, Calcutta.	Asst. Secy., 1939
1934	Mullick, Dr. D. N.	Animal Nutrition Research Institute, Izzatnagar (U. P.).	
1942	Murdoch, Major D., I.M.S.	P. O. W. Medical College, Patna.	
1946	Nath Dr. M. C.	Lecturer in Physiological Chemistry Dacca University.	
1938	Narayana Rai Saheb Prof. B.	P. O. W. Medical College, Patna,	Vice-President, 1943, 1944, 1945, 1946

Year of Election	Name	Address	
1946	Pal Mr. Srikanthabhusan.	76, Bamacharan Chakravarty Road, Dacca.	
1942	Pal, Prof. R. K.	Carmichael Medical College, Calcutta.	Secy. 1938-40
1944	Pillai, Dr. R. K.	Nagercoil, S. India.	
1942	Prasad, Prof. B. N.	Dept. of Pharmacology, P. O. W. Medical College, Patna.	
1942	„ Dr. K. D.	P. O. W. Medical College, Patna.	
1942	Rahaman, Prof. S. A.	Dept. of Physiology, Osmania University, Hyderabad (Deccan).	Vice-President, 1946
1943	Rakshit, Capt. P. C.	63, Townsend Road, Calcutta.	
1942	Ray, Arabinda	Tata Research Scholar, University College of Science, Calcutta.	
1934	„ S. N., I.C.S.	Ballygunj Circular Road, Calcutta.	
1944	Roy, A. C.	Gabtola, Khoshagan, Burdwan.	
1934	„ Dr. B. C.	36, Wellington Street, Calcutta.	
1942	Rudra, M. N.	P. O. W. Medical College, Patna.	
1945	Sadhu Dalalpada	Presidency College, Calcutta.	
1941	Saha, Dr. K. M.	Dept. of Physiology, Carmichael Medical College, Calcutta.	
1944	Sankaran, Prof. G. S.	All India Institute of Hygiene, Calcutta.	Secretary, 1940
1944	Sanyal, Dr. S. C.	73, Vivekananda Road, Calcutta.	
1934	Sarkar, Dr. B. B.	University College of Science, Calcutta.	Treasurer, 1934—36, Secy., —1937-38 Vice-President, 1942, 1943, President, 1944, 1945
1944	Sen, Dr. A. K.	46, Ballygunge Place, Calcutta.	
1944	„ Dr. Barid Baran	Dept. of Physics, University College of Science, Calcutta.	
1934	„ Dr. B. B.	235, Rash Behari Avenue, Calcutta.	
1934	„ P. B.	University College of Science, Calcutta.	
1934	„ Capt. S. K.	43|2, Hazra Road, Calcutta.	
1935	„ Kabiraj S. C.	Kalpataru Palace, Chittaranjan Avenue, Calcutta.	Treasurer, 1934
1934	„ S. C.	Dept. of Physiology, Presidency College, Calcutta.	Treasurer, 1945
1944	Sen Gupta, Dr. S.	P. O. W. Medical College, Patna.	Asst. Secy., 1938, 1942
1942	Shah, Dr. S. S. Mahmud	P. O. W. Medical College, Patna.	
1934	Sil, Dr. K. M.	21, Fordyce Lane, Calcutta.	
1942	Singh, Dr. J. P.	Dept. of Physiology, P. O. W. Medical College, Patna.	
1942	Sinha, Dr. A. S. S.	Dept. of Physiology, Cuttack Medical College, Orissa.	
1942	Swaminathan, Dr. M.	Dept. of Biochemistry and Nutrition, All India Institute of Hygiene.	

The Indian Journal Of Physiology And Allied Sciences

Vol. I JANUARY, 1947 No. I

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Printed and published by
NIBARAN CHANDRA DAS,
Prabasi Press, Calcutta.

THE INDIAN JOURNAL OF PHYSIOLOGY AND ALLIED SCIENCES

JULY, 1947

Published by

THE PHYSIOLOGICAL SOCIETY OF INDIA,

CALCUTTA

Price Rs. 10 per volume.

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The Indian Journal of
Physiology and Allied Sciences
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(From the Department of Physiology, Osmania University Medical College, Hyderabad Deccan).

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Owing to disturbances in Calcutta the publication of the Journal was delayed. It is hoped that things will change for the better in near future and the publication of the jounal could be issued as scheduled.

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The electrocardiograms were taken in the mornings about four hours after the subjects had taken their breakfast. The records were taken with a G. E. Victor Electrocardiograph model A., the subject lying flat on his back. The electrodes used were those supplied by the manufactures of the electrocardiograph. They consisted of curved pieces, 6 cm. by 4 cm. and were evidently made of silver. The area of the skin where the electrodes were applied was cleansed with soap and water and then with spirit. The electrodes were applied to the skin by means of the electrode paste. The record was taken on a 35 mm. film. The instrument was first standardized so that a current of 1 m.v. gave a deflection of 1cm. as determined by pressing the standardization switch. The speed of the film was 25 mm. per second and the accuracy of the speed was frequently checked. The time-marking arrangement consisted of vertical lines across the film at 0.04 second intervals. The electrocardiograms were read with the help of a hand lens.

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Introduction.

Detailed studies of electrocardiograms of normal persons have been made in recent years and workers have obtained in healthy subjects electrocardiographic features which were previously considered abnormal. A study of electrocardiograms from 55 apparently normal Indian subjects was made by 'Telang'. But in view of the recent work done abroad, a detailed study of the electrocardiogram in healthy Indian subjects was considered desirable.

Experimental Subjects and Technique.

The subjects were 75 men, mostly young medical students from seventeen to twenty-five years of age. One of the subjects was aged 32 years and two were over 40 years of age. A careful physical examination was made and only such candidates were selected as were in a good state of health.

Two of the subjects showed sinus arrhythmia but no other defect. None of the subjects showed premature contraction, and type of heart block or any variation in the location of the pacemaker.

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notched, the notch being usually small and present either on the ascent, summit or the descent of the P wave. The notching was most frequent in Lead II. There were often two notches, one on the ascent and the other on the descent of the P wave. Such double notching was more frequent in Leads II & III than in Lead I. In the record of one subject the P wave showed three notches in Leads II & III. Larsen and Skulason[9] have described P waves up to four small notches. Such P waves were not seen in the present series.

In Lead I the average height of the P wave was 0.74 mm., the minimum being 0.2 mm. in four subjects and the maximum being 1.6 mm. in two subjects. In Lead II the average height of the P wave was 1.29 mm., the minimum being 0.2 mm. and the maximum being 2.5 mm. present in only one subject. In the record of three subjects the amplitude of the P wave was 2.0 mm. in Lead II. In all the other subjects the P wave amplitude was less than 2.0 mm. As shown in Table I, in the majority of the subjects Lead II showed the highest P wave. (It may be mentioned here that when the P wave was equally high in two of the leads or in all the three leads, all these leads were counted as showing the highest P wave. It is for this reason that the sum of all the leads with the highest P waves exceeds the figure of seventy-five. The same procedure has been adopted with regard to the other waves of the electrocardiogram).

TABLE I

THE P WAVE.

	Lead.		
	I	II	III
Type of deflection	..		
Positive	75	75	56
Diphasic	0	0	10
Negative	0	0	4
Wavy	0	0	3
Absent	0	0	2
Form			
Notched	49	52	36
Smooth	26	23	28
P wave largest (no. of subjects)	10	62	6
Size (mm.)			
Maximum	1.60	2.50	1.80
Minimum	0.20	0.20	0.20
Average	0.74	1.29	..
Duration (sec.)			
Maximum	0.095	0.120	0.120
Minimim	0.050	0.060	0.050
Average	0.074	0.083	0.076
More than 0.10 sec.	0	1	1
More than 0.08 sec.	4	24	12
P wave longest	33	55	32
P wave 0.08 sec. or more in any lead (no. of subjects)	..	67	..
P wave less than 0.70 mm. in all the leads (no. of subjects)	..	nil	..
P wave less than 1.00 mm. in all the leads (no. of subjects)	..	7	..
The sum of the P waves in all the leads (mm.)			
Maximum	..	5.30	..
Minimum	..	1.02	..
Average	..	2.73	..

The average of the sum of the P wave deflections in all the three leads was found to be 2.73 mm. the minimum being 1.02 mm. and the maximum being 5.30 mm. In no subject was the P wave amplitude less than 0.7 mm. in all the three leads. The height of the P wave, therefore, tallies closely with the results obtained by the American workers.

Duration of the P wave:

The average duration of the P wave in Lead I was found to be 0.074 sec., the minimum being 0.05 sec. in two subjects and the maximum being 0.095 sec. In Lead II the average duration was 0.083 sec., with the minimum of 0.05 sec. and the maximum of 0.12 sec.. In Lead III the results were very much like those of Lead I. The average duration of the P wave was therefore longest in lead II. Only in eight of the subjects was the duration of the P wave less than 0.08 sec. in all the leads. Only in one subject was the P wave in Leads II & III more than 0.10 sec.; it was 0.12 sec. In no subject was the P wave less than 0.07 sec. in all the leads.

These results tally closely with the findings of other workers. Shipley and Hallaran[2] found the duration of P to be between 0.08 & 0.10 sec. in the records of all their 200 normal subjects with the exception of 16 of them. Of these 16, thirteen gave the duration of 0.11 sec. and three gave the duration of 0.12 sec. Ashman and Hull[3] found the duration greater than 0.10 sec. in only one of their 100 records of normal subjects. On the other hand Larsen and Skulason[4] obtained higher results. They found the average duration of the P wave to be 0.10 sec.; the shortest P wave lasted 0.07 sec. and the longest 0.133 sec. In the records of 82 of their 100 subjects, the P wave lasted 0.10 sec. or more. They suggested that, according to their observations, the P wave might be said to be abnormally wide only in cases where the duration was 0.14 sec. or more in at least one of the leads. The age range of these subjects was between 30 and 50 years. Stewart and Manning[4] found the average duration of the P wave in Leads I & III of their 500 subjects to be 0.08 sec. and in Lead II to be 0.09 sec. They calculated a duration of 0.12 sec. as a point at which the P wave duration might be considered as significantly prolonged. Graybiel et al[5] in the records of their 1000 young aviators found the P wave to range from 0.02 sec. to 0.12 sec. in all the leads; their average being 0.074 sec. in Lead I, 0.086 sec. in Lead II and 0.073 sec. in Lead III. These results tally closely with the present observations. Graybiel et al[5] found that P waves of more than 0.10 sec. were only occasionally observed.

The P-R Segment.

The P-R segment (P-Q segment) signifies that portion of the electrocardiogram which lies between the end of the P wave and the beginning of the QRS Complex. Table II shows that in the vast majority of cases (about 75% or more) the course of the P-R segment was horizontal and in most cases it was iso-electric. In very few cases it was above the iso-electric lines. In a few cases it had a rising course in Leads II and III but in no case it had a rising course in Lead I. The maximum deviation from the iso-electric line in either direction was 0.7 mm. and this occurred in Lead II.

At present no clinical significance is attached to this deflection. Larsen and Skulason[6] found the maximum deviation of the P-R segment from the iso-electric line in either direction to be 0.5 mm. According to Pardee[7] it is occasionally found in abnormal cases to be as much as 2.5 mm.

TABLE II

The P-R Segment and the P-R Interval.

	Lead.		
	I	II	III
Course of P-R segment (no. of subjects)			
Horizontal	59	57	58
Falling	16	11	9
Rising	0	7	8
Iso-electric	52	51	58
Above iso-electric line	0	2	4
Below iso-electric line	23	22	13
Duration of P-R interval (sec.)			
Maximum	0.200	0.220	0.220
Minimum	0.100	0.100	0.100
Average	0.137	0.145	0.142
0.20 sec. or more (no. of subjects)	1	1	1
0.18 sec. or more (no. of subjects)	2	5	3
Less than 0.12 sec. (no. of subjects)	2	3	0
P-R longest (no. of subjects)	35	51	36

The P-R Interval.

The P-R interval signifies the time from the beginning of the P wave to the beginning of the QRS complex. It is also called the P-Q interval but, as the Q wave is frequently absent, the term P-R interval is usually applied. This interval is a measure of the time taken for the activation process (impulse) leaving the sinus node to reach the ventricles. It is a measure of auriculo-ventricular conduction time.

The P-R interval as fixed by the American Heart Association is from 0.12 to 0.20 sec.

In the present series, taking all the three leads into consideration, the average duration of the P-R interval was found to be 0.141 sec.; the shortest duration was 0.10 sec. and the longest duration was 0.22 sec. which occurred in only one subject. In no subject was the duration of the P-R interval less than 0.12 sec. in all the leads. The duration of the P-R interval was usually the longest in lead II as shown in Table II. These results tally closely with those given by Larsen and Skulason[6]. One may therefore consider a P-R interval of 0.22 sec. as being within the normal range.

It is interesting to note that Stewart and Manning[4] reported four young men whose electrocardiograms had P-R intervals of more than 0.24 sec. They were apparently normal men with no abnormality except the long P-R conduction time. The average duration obtained by these workers in the records of their 500 subjects was 0.15 in Leads I & III & 0.16 in Lead II. Graybiel et al[5] in a series of 1000 subjects found the P-R interval to vary in duration from 0.09 sec. to

0.28 sec., the average being 0.154 sec. They found four cases where the P-R interval was 0.21 sec. and 8 cases where it was 0.22 sec. and one case each where it was 0.24, 0.25, 0.26 & 0.28 sec. Careful examination in these subjects did not disclose any infection or other abnormality. But the cases were not under observation for more than a few days. Graybiel et al' describe a relationship between the P-R interval and the cardiac cycle, the P-R interval varying with the length of the cardiac cycle. They did not find the P-R interval longer than 0.20 sec. where the heart rate was 80 or above. It is interesting to note that in one of the subjects of the present series whose records showed a P-R interval of 0.22 sec., the heart rate was as high as 88.

The QRS Complex.

The QRS complex was usually diphasic or triphasic. Only in a few cases was it monophasic. In no case was the R wave absent. Monophasic QRS complexes occurred only two times in Lead I, six times in Lead II and four times in Lead III.

The Q wave:

Table III shows that the Q wave was present in about 50% of the cases in every lead. It was slightly more frequent in Lead II, being present in 46 of the cases. The maximum amplitude of the Q wave in Lead I was 2.0 mm. and in 10 cases Q_1 was more than 1.0 mm. in amplitude. Table IV shows that the Q waves were present in most of the cases and that they frequently occurred simultaneously in more than one lead.

Q1 was never more than 2.0 mm. and this amplitude occurred only in 2 cases. Only in one case was Q1 greater than 15% of the highest wave in any lead. In two subjects Q2 was greater than 20% of the highest wave of QRS in any lead. In one of these two subjects Q3 was 4.4 mm. and was more than 25% of the highest wave in any lead. These subjects had electrical axes within normal

TABLE III

The QRS Complex

	Lead.	
Q wave	II	III
Present (no. of subjects)	38	
Maximum (size in mm.)	2.0	3.0
R wave (*size in mm.*)		
Maximum	12.20	
Minimum	2.10	6.00
Average	5.52	10.70
Less than 5 mm. (no. of subjects) ..	26	1
R wave largest (no. of subjects) ..	5	71

TABLE III—*contd.*

	Lead. I	II	III
S wave			
Present (no. of subjects)	61	60	51
Maximum (size in mm.)	2.00	4.00	4.40
Duration of QRS Complex (sec.)			
Maximum	0.100	0.100	0.100
Minimum	0.050	0.060	0.060
Average	0.074	0.080	0.080
0.08 sec. or more (no. of subjects)	40	52	52
0.10 sec. (no. of subjects)	2	3	4
Less than 0.06 sec. (no. of subjects)	3	0	0
Less than 0.08 sec. in all the leads (no. of subjects)	..	11	..
The sum of R waves in all the leads (mm.)			
Maximum	..	39.0	..
Minimum	..	13.2	..
Average	..	23.4	..
Less than 15 mm. (no. of subjects)	..	4	..
The sum of QRS waves (mm.)			
Maximum	13.3	24.6	20.5
Minimum	2.2	3.1	3.5
Average	7.60	13.46	9.87
Total of the sum of QRS waves in all the leads (mm.)			
Maximum	..	53.7	..
Minimum	..	20.8	..
Average	..	31.0	..
Less than 25.0 mm. (no. of subjects)	..	13	..
R wave			
Less than 5 mm. in all the leads (no. of subjects)	..	nil	..
Less than 7 mm. in all the leads (no. of subjects)	..	3	..

limits. Thus Q waves exceeded the limits given by Kossman, Shearer and Texon[6] for normal absolute and relative sizes of the Q waves in records from 178 healthy young people and found the normal maximum values of 2.0 mm. in Lead I, 2.5 mm. in Lead II & 3.0 mm. in Lead III.

According to the Criteria Committee of the New York Heart Association[7] a Q wave is considered abnormally deep if Q1 exceeds 15% of the largest QRS deflection in any lead or Q2 exceeds 20% or Q3 25%. These criteria do not hold if QRS exceeds 20 mm. or for Leads II & III in presence of right axis deviation.

Stewart & Manning[4] in the records from 500 subjects, found that six records had Q1 waves which were more than 15% of the maximum R, two Q2 with more than 20% of maximum R and ten Q3 with more than 25% of maximum R amplitude. Out of these, four records were excluded for showing right axis deviation. The remaining twelve had normal axis deviation and no other significant electrocardiographic abnormalities and the men were clinically normal in all respects.

Graybiel et al[5] found three instances where Q1 was 20% or more of the tallest R in any lead. The maximum amplitude of Q2 was found to be 3.5 mm.

A QRS complex lasting 0.10 to 0.12 sec. is characterized as prolonged and has been regarded by some as an instance of incomplete bundle-branch block. According to Larsen and Skulason[6], who found in 14 of their subjects a duration of QRS of 0.10 sec. or more, QRS complexes lasting from 0.10 to 0.12 sec. have to be looked upon as normal. Stewart & Manning[4], in the aecords obtained on 500 subjects, did not find the duration of QRS complex to exceed 0.10 sec. in Lead I, but five exceeded this time in Lead II and six in Lead III. They considered a QRS complex of more than 0.12 sec. duration to be definitely abnormal. Graybiel et al found 27 instances where the QRS measured 0.11 sec. and eight instances in which the duration was 0.12 sec. There was no other abnormality in the electrocardiogram and the heart was apparently healthy in these subjects. They concluded that a duration of 0.11 sec. was sufficiently frequent in the records of electrocardiogram for young healthy persons and was therefore not necessarily pathological. A QRS complex of 0.12 sec. duration might, in rare instances, not have any pathological significance.

The Electrical Axis:

The Criteria Committee set 30 to 90 degrees as the normal range of the electrical axis while Ashman & Hull[3] defined the limits of normality as 40 to 90 degrees. White[10] defined the range of normality from 0 to 90 degrees.

The electrical axis in the present group was measured according to the method of Carter, Richter & Greene[11]. The range was from—37° to +102°, the average being 60°. In three subjects (4%) the electrical axis was less than zero and in two subjects it was more than 90 degrees. Thus in only 5 cases the electrical axis fell beyond the range specified by While. In eight subjects (11%) the axis was less than 30 degrees which, according to the standards of the American Heart Association, was enough to classify them under left axis deviation.

In the 100 subjects of Larsen and Skulason[6], left preponderance was found in five cases though the roentgenoram did not show any pronounced transverse position of the heart. The measurement of the left preponderance was done according to the rule made by one of them which required that the S wave in Lead III exceed the R wave in the same derivation as to size and duration and also that it be greater than one-half of the R wave in Lead I. In 18 subjects they found a slight degree of left axis deviation, a deviation which would fall between 0° to 30°. All except four of those subjects were over 40 years of age. Larsen & Skulason concluded that left preponderance and a tendency towards it were considerably more frequent in the age class of 40 to 50 than in the age class of 30 to 40 years. In nine of these eighteen subjects the weight was above the normal average. They did not have any instance of right preponderance in their subjects.

Stewart and Manning[4], in the electrocardiograms of their 500 subjects, found 22 cases (44%) with electrical axes of less than 0°, whereas 42 subjects had axes over 90°.

Graybiel et al[5] found the angle of the electrical axis, calculated according to the method of Carter, Richter & Greene, to range from +120° to —36° and the mean angle was 64.2°.

It is thus seen that in the present series, as in the case of records obtained by other observers, there are many young persons without hypertension and with apparently normal sized heart where the angle of the electrical axis falls beyond the "normal" range of 0°..90°.

Notching & Slurring

Lead I did not show any notching. In six cases there was slurring of R wave in Lead I, out of which in two cases the slurring was apical (above the middle of the R wave), the height of the R wave being more than 6 mm. In four cases the slurring of the R wave was basal (in the middle or in the lower regions of the R wave). In one of these cases basal slurring of both the R and the S waves was present.

Slurring of the R wave in Lead II was seen in 9 cases out of which in 2 cases the slurring was apical. In one subjects both apical and basal slurring was present in the descending limb of the R wave the height of which was 12 mm. In the rest of the 9 cases, the slurring was basal of which in two there was basal notching. In four cases there was slurring or notching of the S wave in Lead II. In one case, basal slurring of the R wave was present together with notching of the S wave. In three cases basal slurring of the R wave in Lead I was present along with slurring or basal notching of the R or the S wave in Lead II. In no case was there an apical slurring of the R wave in Leads I & II together.

Both notching and slurring were very frequent in Lead III, about half of the subjects showing such irregularity. In three cases the QRS was vibratory (More than triphasic). In two cases the R wave in Lead III showed a high take-off in the descending limb.

There is still some confusion as to the significance of notching & slurring. Though notching or slurring is common at the beginning or at the end of the QRS complex, it is infrequent during the middle or top of a large excursion of QRS in Leads I & II. According to Pardee[7], notching or slurring in the course of one peaks of QRS can be considered normal only when it occurs in one lead alone, and in Lead I when that lead has a small excursion of QRS. According to the Criteria Committee of the New York Heart Association[9], notching or slurring or localized splintering is normal if confined to one lead only, or in more than one lead if confined to apex or base in such other leads.

In the present series no abnormal notching was present as defined by the above mentioned standards. But notching or slurring which would be abnormal according to the above-mentioned standards have been recorded by other workers as occurring in subjects with hearts which were clincally normal. Stewart & Manning found two records showing slurring & splintering in all the three leads and one record showing these features in Leads II & III. This irregularity occurred nearer the apex of QRS in some leads than in others of the same record